RELATIONSHIP OF HYPOTHALAMIC CATECHOLAMINE ALTERATIONS AND REPRODUCTIVE FUNCTION IN AGING FEMALE RATS

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KEY OF ABBREVIATIONS

AP	anterior pituitary
ARC	area retrochiasmatica
CA	catecholamine
CE	constant estrous
CLON	clonidine
CV	coefficient of variation
DA	dopamine
DBH	dopamine-β-hydroxylase
DDC	diethyldithiocarbamate
EDTA	ethylenediamine tetraacetic acid
F344	Fischer 344
FLA-63	bis(4-methyl-1-homopiperanzinyl thiocarbonyl)disulfide
IC	irregularly cycling
K	rate constant of amine loss
L-DOPA	dihydroxyphenylalanine
LH	luteinizing hormone
LHRH	luteinizing hormone releasing hormone
LSD	least significant difference
MBH	medial basal hypothalamus
ME	median eminence
MFB	medial forebrain bundle
αMPT	α-methylparatyrosine

NA	nucleus arcuate
NAc	nucleus accumbens
NC	normally cycling
NE	norepinephrine
NHA	anterior hypothalamic nucleus
NSC	nucleus suprachiasmatica
NSO	nucleus supraoptica
NVM	nucleus ventromedialis
OVLT	organum vasculosum of the lamina terminalis
POA	preoptic area
POAm	preoptic area medialis
POAs	preoptic area suprachiasmatica
PP	repeated pseudopregnant
SCN	suprachiasmatic nucleus
SON	supraoptic nucleus
RIA	radioimmunoassay
TIDA	tuberinfundibular dopamine
TRH	thyrotropin releasing hormone
VMN	ventromedial nucleus

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This work has examined the relationship between age-related changes in hypothalamic catecholamine (CA) neurons and impaired gonadotropin regulation in female rats which exhibit two patterns of reproductive senescence. Long-Evans and Fischer 344 (F344) rats which exhibit the constant estrous (CE) and repeated pseudopregnant (PP) states respectively, during advanced age were studied. Norepinephrine (NE) and dopamine (DA) turnover rates were examined in microdissected hypothalamic regions in young, middle-aged and old ovariectomized Long-Evans and F344 rats to characterize the pattern, extent and loci of age-related changes in CA neuronal function. Endocrine and pharmacological manipulations were employed to evaluate the contribution of the described changes in CA activities to altered regulation of luteinizing hormone (LH), prolactin and LH releasing hormone (LHRH) during advanced age.

Results of these studies show that no widespread progressive decreases in DA or NE neuronal function accompany the aging process. Age-related changes in magnitude and direction within and between F344 and Long-Evans

rats. While CA concentrations generally decrease during advanced age, CA turnover rates are maintained or augmented in several hypothalamic regions of older compared to younger animals, particularly in long-lived F344 rats. Hyperprolactinemia appears to maintain the PP state in old F344 rats and may be responsible for increased DA turnover in several hypothalamic regions during aging. Decreased postcastration LH response in CE rats results from impaired LHRH neuronal function as reflected by decreased frequency and amplitude of LH pulses. An α-adrenergic agonist can restore pulsatile LH release in both ovariectomized young rats with induced NE depletion and in middle-aged CE rats in which decreased NE activity was observed, but not in old CE rats. Thus, adrenergic dysfunction appears to mediate the onset of the CE state, while subsequent alterations may maintain the CE state. These results show that complex patterns of CA neuronal change contribute to impaired hormone regulation during advanced age and suggest that pharmacological agents may be employed to improve age-related neuroendocrine dysfunction.

INTRODUCTION

Advanced postmaturational age is associated with a generalized decline of many physiological functions. Although several hypotheses have been proposed to explain age-related impairments in regulatory processes, the mechanisms responsible for these alterations remain poorly understood. Due to the central role of the neuroendocrine system in maintaining homeostasis through regulation of the hormonal milieu, changes in this system with age could potentially be responsible for many age-related impairments. This concept predicts that changes in hormone regulation occur during advancing age and further suggests that age-related impairments might be corrected by restoring or maintaining neuroendocrine regulatory function.

Among the most recognized age-related changes in physiological functions is the decline of reproductive capacity and cessation of regular ovulatory cycles. While the menopause in middle-aged women appears to result primarily from ovarian oocyte exhaustion, altered gonadotropin regulation appears primarily responsible for demise of reproductive function in female rats. Similarly, altered gonadotropin levels have been associated with impaired reproductive activity in aged men and male rats. Aging female rats exhibit various patterns of reproductive senescence which appear to result from alterations in neuroendocrine regulatory functions. Most rats have irregular and lengthened ovarian cycles in middle-age prior to establishment of noncyclic reproductive states. Following a variable period of irregular cycles many rats enter a constant estrous (CE) noncyclic state. Constant estrous rats have well developed follicles which

secrete moderate levels of estrogen, but fail to secrete sufficient luteinizing hormone (LH) to stimulate ovulation. A hypothalamic alteration appears to prevent the normal steroid induced release of LH in these CE animals. Other rats enter a repeated pseudopregnant (PP) state in later life. In this PP state animals ovulate at 8 to 20 day intervals with corpora lutea maintained and elevated progesterone secretion between ovulatory events. Old PP rats appear to have relatively normal LH response to a stimulatory regimen of gonadal steroids. Some old rats become anestrous with atretic ovaries and have chronically elevated prolactin levels. This state appears to result from a pathological condition at the level of the anterior pituitary (AP) characterized by a relative absence of functional gonadotrophs and proliferation of mammotrophs.

Interestingly, the PP state appears to be associated with longer lived rats compared to the CE state. This observation together with the demonstrated difference in ability of the neuroendocrine system of these two animal models to respond to stimulatory regimens of gonadal steroids with LH secretion is consistent with the concept that alterations in the neuroendocrine system may be responsible for many age-related declines in physiological function. However, the nature of alterations which result in the onset of the CE or PP state in aging rats is largely unknown. Elucidation of changes in hormone regulation which occur in these animal models would provide a better understanding of mechanisms responsible for age-related alterations in homeostasis and perhaps suggest approaches to treat these impairments.

Neuroendocrine systems can broadly be described as transducer systems which convert neural signals into endocrine output. The importance of neuroendocrine regulation of reproductive function has been identified

by numerous investigators through a variety of experimental approaches. The secretion of AP hormones is regulated by neurohormones produced in the hypothalamus and transported to the AP via the hypophysial portal system. Inputs which modulate activity of neurohormone secretion of hypothalamic neurons have been extensively studied. These inputs consist of synaptic transmission signals from putative neurotransmitters. Although age-related alterations in neurotransmitter activities have been reported in several brain regions, the extent and rate of these changes on reproductive function in rats are not well characterized. Experiments described in this thesis examine the activity of luteinizing hormone releasing hormone (LHRH) neurons and activity of the neurotransmitters, dopamine (DA) and norepinephrine (NE), within hypothalami of aging rats which enter the CE or PP state. These studies are designed to evaluate the extent and identify the locus of neuroendocrine alterations which occur in the CE and PP states. In addition to identifying the locus of age-associated alterations in the neuroendocrine system of CE and PP animals, studies are described which evaluate the effects of manipulation of neurotransmitter systems on LH secretion in old animals.

It is hoped that the results of studies described in this thesis will improve our understanding of the mechanisms responsible for age-associated declines in hormone regulation by (i) more clearly identifying central loci responsible for altered gonadotropin secretion (ii) permitting quantification of the consequences of altered neuron function on gonadotropin secretion and, (iii) evaluating the potential for pharmacological correction of age-related alterations in neuroendocrine function.

LITERATURE REVIEW

Hypothalamic Control of Anterior Pituitary Function

Hypophysiotropic Hormones

The concept that neurohormones regulate AP secretory function evolved from a variety of experimental observations and several experimental approaches conducted by numerous investigators. McCann and Porter[1] reviewed early studies which led to this concept.

The close anatomical proximity of the AP and hypothalamus suggested to Popa and Fielding that the portal vasculature connecting these tissues served to transport hormones from the AP to the hypothalamus.[2] A few years later, the direction of blood flow was shown to course from the brain to the AP by following the movement of dyes in the portal blood.[3] The Scharrers first proposed and later demonstrated that hypothalamic neurons secreted hormones.[4,5] This concept led Harris to propose the "chemotransmitter hypothesis" which essentially stated that neurosecretory products from hypothalamic neurons traveled via the hypophysial portal system to regulate hormone secretory activity of the AP.[6]

Early evidence supporting this hypothesis came from studies which lesioned the hypothalamus or severed the connections between the hypothalamus and pituitary. These procedures resulted in atrophy of the gonads, thyroid and adrenal glands (see 1). Similarly, removing the pituitary from the sella turcica and transplanting it to the anterior chamber of the eye or under the kidney capsule resulted in a variety of metabolic changes including atrophy of endocrine glands stimulated by hypophysiotropic hormones. However, ectopic pituitaries maintained corpora

lutea[7] and mammary gland function.[8] These experiments indicated the central nervous system (CNS) exerted both a stimulatory and an inhibitory effect on AP hormone secretions.

Another approach used in early investigations to confirm the "chemotransmitter hypothesis" used electrical stimulation of hypothalamic regions. Harris and coworkers showed in a series of studies that hypothalamic stimulation could induce ovulation in rabbits,[9] increase adrenal activity,[10] and increase thyroid weight.[11]

A classic approach used by endocrinologists when attempting to identify the origin and characteristics of a putative hormone is to study the effects of various types of extracts made from the tissue suspected of secreting the substance. Results of studies utilizing this approach during the late 1950's and 1960's demonstrated hypothalamic releasing hormone activity for each of the AP hormones and hypothalamic inhibiting activity for prolactin and growth hormone (see 1, 12). The effects of hypothalamic extracts were generally negated when extracts were treated with trypsin which indicated the putative neurohormones were peptides or proteins.

The structural elucidation of neurohormones was and continues to be of major interest to investigators who examine CNS regulation of AP function. Successful identification and synthesis of four neurohormones during the last decade have vastly improved understanding of neuroendocrine regulatory mechanisms. Synthesis of these hormones permitted development of both radioimmunoassay (RIA) and immunohistochemical techniques to quantify these hormones as well as to facilitate studies designed to characterize the physiological function of these substances. The first releasing factor to be identified was thyrotropin releasing hormone (TRH).[13, 14]

The tripeptide has identical structure and releases thyrotropin in all species which have been examined. Thyrotropin releasing hormone also stimulates prolactin secretion in rats, cows and humans.[15-17] Two years after the identification of TRH, the ten amino acid sequence of LHRH was elucidated in bovine[18] and porcine[19] hypothalamic extracts. Extensive studies of LHRH have shown it has identical structure and similar ability to release both LH and follicle stimulating hormone in all species tested. Growth hormone inhibiting hormone (somatostatin) was identified and synthesized in 1973.[20] Recently, a 41 amino acid sequence with corticotropin releasing activity has been characterized.[21]

Extensive study of these four peptide structures confirmed their roles as neurohormones through meeting the criteria proposed by McCann and Porter to prove neurohormone function. These criteria include

1. demonstration of secretory elements in the brain which produce the putative neurohormone within diffusable distance from the portal vessels,
2. identification and characterization with biological, physical and chemical methods of the putative neurohormone within portal vessels,
3. demonstration that the putative substance alters AP hormone release after entering the portal vessels.[1]

Conclusive identification of other hypothalamic substances as neurohormones with these criteria awaits further study.

Hypothalamic Anatomy

General. The hypothalamus is the most ventral portion of the diencephalon and is partially exposed on the ventral surface of the brain. Hypothalamic structures evident on the ventral surface (rostral to caudal) are the optic chiasm, infundibulum, tuber cinerium and mammillary bodies.

The base of the infundibulum together with the rostral portion of the tuber cinerium is the median eminence (ME). Anatomically, the hypothalamus is defined dorsally by a groove in the wall of the third ventricle called the hypothalamic sulcus; rostrolaterally by the first segment of the optic tract; caudolaterally by the groove formed by the cerebral peduncles; and, caudally by the limiting border of the mammillary bodies.[22] The rostral border is indistinctly separated from the preoptic area (POA) and the POA is sometimes included as part of the hypothalamus.[23] The optic chiasm and lamina terminalis are generally considered to form the rostral most hypothalamic border.[22] The hypothalamus is often divided into four gray areas: the POA, the anterior hypothalamic region, the medial hypothalamic area and the posterior hypothalamus. It should be noted that these regions are arbitrarily defined since several hypothalamic nuclei occupy more than one region. The ME is often considered as a separate region.

Vascular supply. While all of the hypothalamus is vascularized from the ventral surface via branches from the Circle of Willis, the five hypothalamic regions each receive blood from separate arteries of this system and are generally thought to have relatively separate venous drainage. The vascularization of each region has been discussed in detail by Ambach and Palkovits.[23] Of special interest is the vascularization of the ME region. The ME and nucleus arcuate (NA), which lies dorsal to the ME, are richly and exclusively supplied by hypophysial arteries. These arteries form a capillary network in the ME by multiple anastomoses called the primary plexus of the ME or palisadic zone. The ME arterial supply contains fenestrated capillaries characteristic of the circumventricular organs and therefore the ME is outside of the blood-brain barrier. Veins collect in several directions from these capillaries to form a portal

circuit of the AP. Portal veins of the AP collect blood from the rostral, dorsal and lateral portions of the ME and branch into a secondary capillary plexus in the pituitary.

Hypothalamic nuclei. Anatomically, the nuclei within the hypothalamus are often less distinct compared to other brain regions such as the thalamus. Since a brain nucleus is defined as an area where the density of cells is higher than in surrounding areas, boundaries between nuclei are usually identified on the basis of relative position of cellular densities.[24] Hypothalamic nuclei are bilaterally distributed along either side of the third ventricle with the exception of the ME and organum vasculosum of the lamina terminalis (OVLT). The OVLT, like the ME, is a circumventricular organ.

Although nuclear regions within the hypothalamus were identified by early anatomists, Palkovits most extensively characterized the location and size of more than 20 specific hypothalamic nuclei in the rat.[24] Concomitant with his anatomical description, he developed a microdissection or "punch" technique which has been used extensively to identify the potential neuroendocrine function of many hypothalamic nuclei.[24]

The importance of the hypothalamus in maintaining homeostasis was recognized from numerous early studies which employed both lesion and stimulation techniques. In addition to regulating AP secretory activity, the hypothalamus regulates appetite, water balance, body temperature, blood pressure, the sleep-wake cycle and behavior. Although the known functions of hypothalamic regions have been reviewed extensively,[25] the identification of the anatomical loci associated with each of these functions has been, and continues to constitute, a major aspect of neuroscience research.

The medial base hypothalamus (MBH) was first identified as the region of primary importance in regulating AP hormone secretion by Halász and co-workers.[26,27] They noted that AP tissue was able to maintain integrity of the thyroid, adrenals and gonads when it was transplanted to the MBH of rats but the AP and its target tissues degenerated when it was implanted in other brain regions.[26] Halász and Pupp severed the "hypophysiotropic" region from other brain regions in rats and observed that the AP histology and thyroid and testicular weights were maintained, but the ovaries and uterus were atrophied.[27] Further studies showed that the neural connection between the POA and MBH is required for cyclic release of gonadotropins in rodents, but does not appear essential in primates (see 28).

The majority of evidence now indicates that hypothalamic regulation of basal gonadotropin secretion originates in nuclei of the MBH while the ovulatory hypersecretion of LH or "cyclic release" is mediated by more rostral hypothalamic regions in rodents (see 29). Of the nuclei located between the POA and MBH, the suprachiasmatic nucleus (SCN) appears important in timing LH hypersecretion with diurnal photic cycles.[30]

Innervation of the Hypothalamus

Afferent and Efferent Pathways

The hypothalamus receives afferent and sends efferent projections through a variety of pathways to communicate with many brain regions as might be expected from its diverse regulatory functions. Evidence for seven major fiber tracts integrating the hypothalamus with other brain regions has been reviewed by Palkovits and Zaborszky.[31] The medial forebrain bundle (MFB) is a diffuse fiber tract which courses through the lateral hypothalamus. Descending afferents from the MFB to the hypothalamus originate in the olfactory bulbs, septum, pyriform cortex and nucleus

accumbens (NAc). Projections from the raphé nuclei and dorsal and ventral tegmental nuclei ascend via the MFB. Both ascending and descending MFB projections exchange afferent and efferent fibers most densely in the medial portion of the hypothalamus as well as innervating the POA regions. The fornix provides afferents to the mammillary bodies from the hippocampus. The medial corticohypothalamic tract diverges from the fornix to innervate the periventricular, SCN and MBH regions of the hypothalamus with hippocampus projections. The stria terminalis originates in the amygdala and projects to the POA and MBH regions. Three nerve tracts course primarily through the mammillary bodies: the mammillothalamic tract, mammillotegmental tract and the mammillary peduncles. The first two do not involve major connections to other hypothalamic regions, while the last connects the posterior and lateral hypothalamus with the ventral tegmental area.

Noradrenergic Pathways

The role of NE as a putative neurotransmitter was first suggested by Vogt[32] who observed high concentrations of the catecholamine (CA) in the brain and its differential distribution among brain regions. Subsequent development of histofluorescent and radioenzymatic techniques in the late 1960's and early 1970's demonstrated that the hypothalamic neuronal network is rich in CAs. Experimental evidence leading to elucidation of the CA containing pathways to the hypothalamus and detailed descriptions of these pathways have been extensively reviewed.[25,31,33-35]

Norepinephrine pathways in the brain originate in cell bodies located in the lower brain stem. No NE-containing perikarya have been detected in the hypothalamus. The cell bodies are located in five major clusters: the lateral reticular nucleus (A1 cell group), solitary tract nucleus

(A2 cell group), ventrolateral pontine nucleus (A5 cell group), locus ceruleus (A6 cell group) and mesencephalic reticular formation (A7 cell group). All NE cell groups participate in hypothalamic innervation through three fiber tracts: the ventral NE bundle and the ventral and dorsal periventricular NE bundles. The dorsal NE bundle which originates primarily in the A6 cell group sends projections to the dorsal hypothalamic, periventricular and paraventricular nuclei via branches to the central NE and dorsal periventricular NE pathways. The majority of NE fiber projections to the hypothalamus course through the ventral NE bundle which contains axons from cell bodies located primarily in the A1 cell groups, although each NE cell group contributes to the bundle. At the rostral mesencephalic region, the ventral NE bundle receives fibers from the dorsal NE bundle and then joins the MFB. The ventral periventricular NE bundle is formed from medial projections of MFB fibers at its entrance to the hypothalamus. Other fibers from the MFB diverge at the lateral hypothalamus and terminate in the MBH while the majority of fibers course further rostrally. At the posterior border of the optic chiasm, fibers from the MFB branch through the lateral retrochiasmatic region and enter the MBH from the rostral and lateral aspects.

The external source of hypothalamic NE pathways has been confirmed by studies which severed the regions from other brain areas. Deafferentiation of the medial hypothalamus resulted in a 70-90% NE depletion in this region.[36,37] The residual 10-30% of NE may be present in neuroglial cells and thus be unresponsive to lesion of NE fiber tracts.[38] Similar approaches which lesioned specific NE cell groups in the brain stem resulted in general NE reductions in all hypothalamic areas; however, the rate of NE depletion within hypothalamic regions varied depending on which

brain stem regions were lesioned.[39] The depletion in each case was not as great as complete hypothalamic deafferentiation. The dense innervation network in the hypothalamus and results of lesion studies of brain stem cell groups suggests axon collaterals originating from other NE cell groups may compensate to innervate all hypothalamic regions when the primary source of NE to a particular region is lost.[35] Thus, the specific primary source of NE input to each hypothalamic area has not been clearly established.

Dopaminergic Pathways

Unlike NE, DA innervation of the hypothalamus originates from both extrahypothalamic and intrahypothalamic pathways. Intrahypothalamic sites of DA perikarya include the NA (A12 cell group), hypothalamic and preoptic periventricular nuclei (A14 cell group) and zona incerta area of the dorsomedialis subthalamus which extends to the adjacent ventral periventricular nuclei (A13 cell group). Extrahypothalamic sources of DA originate in cells located in the pars compacta of the substantia nigra and the ventral tegmental area. Catecholamine-containing perikaryia within these regions are often designated as A8, A9 and A10 cell groups, although the anatomical distinction between DA containing cell groups is not clear.[34] Projections from these mesencephalic regions form two major DA systems. The nigrostriatal DA system arises primarily from the pars compacta of the substantia nigra and projects rostrally via a pathway which includes the lateral hypothalamus to terminate in the neostriatum (caudate putamen) and glabus pallidus. The mesocorticol-mesolimbic DA system arises primarily from the ventral tegmental region and ascends rostrally close to the nigrostriatal fibers. Four major branches from the ascending mesocorticol pathway form projection routes which terminate

in the exocortex, and allocortex. The allocortex includes the olfactory, bulb and tubercle, periform cortex, NAc and amygdala or amygdaloid areas.

Two intrahypothalamic and one extrahypothalamic DA pathways have been described. The tuberoinfundibular DA (TIDA) pathway consists of short axons which course from DA cell bodies in the NA (A12 cell group) and project to the ME. The majority of DA in the ME originates from cells located in the NA. Axons from the TIDA system have been shown to terminate from the ME to the caudal end of the infundibulum, although some evidence indicates cells from the rostral portions of the TIDA system send axons caudally to innervate the posterior pituitary gland.[34] Additionally, projections from the A12 cell group are thought to terminate in the NA, ventromedial nucleus (VMN) and premammillary nuclei since deafferentiation of the MBH which includes these areas fails to alter DA concentrations in these nuclei.[36] Axon collaterals from fibers running into the ME are thought to innervate these three nuclei.[35] The incerto-hypothalamic DA system originates primarily in the A13 cell group but also receives contributions from some axons originating in the A10 and A11 cell groups. Fibers from this system run periventricularly in the dorsal hypothalamus and have been detected to course rostrally to the POA-hypothalamic border. The incerto-hypothalamic DA system has been shown to innervate the dorsomedial and paraventricular nuclei;[40,41] however, its role in AP hormone regulation has not been demonstrated.[42] Axons originating from cell bodies in the A14 cell group innervate the medial preoptic nucleus (POA_m) and periventricular nucleus. Dopamine sources from extrahypothalamic regions enter the hypothalamus via the mesocortical DA pathway. Deafferentiation of the hypothalamus fails to substantially alter intrahypothalamic DA levels indicating this pathway does not constitute the primary

DA innervation to the hypothalamus. However, lesion of A8, A9 and A10 cell groups decreases ME levels of DA and results in degenerated nerve terminals in the external layer of the ME.[43,44] The origins of DA innervation of the supraoptic nucleus (SON), anterior hypothalamic nucleus (NHA), SCN, and posterior hypothalamic nuclei are not yet conclusively identified.[35]

Other Putative Neurotransmitter Pathways

In addition to the high concentrations of CA found in the hypothalamus relative to other brain regions, several other putative neurotransmitters are localized in this region. Pathways of these substances have been reviewed.[31,35] Epinephrine is found in axons terminating in the dorsomedial, paraventricular, periventricular, NA and SON hypothalamic nuclei. Cell bodies for epinephrine axons are located in the C1 and C2 regions of the medulla oblongata; however, the pathways by which these fibers reach the hypothalamus have not been demonstrated.[35] Serotonin innervation to the hypothalamus arises from the narrow band of midbrain raphé nuclei and projects via the MFB to terminate in the SCN as well as other hypothalamic and POA regions.[31] Although most serotonin appears to arise from extrahypothalamic origins, some evidence indicates the dorsomedial nucleus may contain serotonin perikarya.[45] Histamine is present in all hypothalamic regions and hypothalamic deafferentation does not greatly reduce histamine levels in the MBH.[31] These results have been interpreted to indicate histamine may be located in nonneuronal cells.[46] Similarly, deafferentation does not change MBH concentrations of acetylcholine nor choline acetyltransferase.[31]

Catecholamine Regulation of Luteinizing Hormone

Initial Evidence

A role for CA in the regulation of LH release was initially proposed by Sawyer and colleagues who observed intraventricular injection of NE resulted in ovulation in estrous rabbits.[47,48] Since NE did not alter LH release *in vitro*, a central mechanism for NE action was proposed.[49] The resulting hypothesis that noradrenergic neurons exert a stimulatory influence on LH release has been supported by a variety of experimental approaches which have been extensively reviewed.[49,50]

Anatomical Evidence

Anatomical evidence is presently insufficient to state the precise nature of CA and LHRH neuron connections; however, high densities of CA nerve terminals and LHRH containing cell bodies have been histologically identified in close synaptic-like proximity in the ME.[51] Early immunohistochemical studies failed to conclusively identify LHRH containing perikarya (see 50); however, more recent studies showed positive staining LHRH perikarya in the POA and SCN regions as well as the NA-ME areas.[52] This evidence, together with the observation that LHRH in the MBH is depleted following anterior deafferentation,[53] supports the existence of a preopticoinfundibular LHRH pathway proposed by Everett as a result of electrophysiological studies.[54]

Electrophysiological studies demonstrated electrical stimulation of the POA_m results in altered multiple unit activity in the NA and ME in rats and increased LH release.[54,55] Further, the magnitude and duration of the stimulus were somewhat proportional to the amount of LH released.[56] Since intraventricular injection of NE resulted in similar alteration in multiple unit activity in the ME[57] and augmented LH release in a dose

dependent manner,[58] correlative evidence implicated that NE stimulates the preopticoinfundibular LHRH pathway.

Biochemical Evidence

A biochemical approach has been to examine CA metabolism during physiological states of increased LH secretion. Following ovariectomy, anterior hypothalamic NE concentrations increase,[59] the rate of tritiated-NE depletion increases in whole brain,[60] hypothalamic NE turnover is augmented,[61] and NE synthesis from tritiated-tyrosine increases.[62] Ovariectomy also augments the activity of the rate limiting enzyme in CA synthesis, tyrosine hydroxylase.[63] Additionally, the preovulatory discharge of LH as well as the gonadal steroid induced surge of LH in ovariectomized rats is preceded by increased NE turnover which reflects augmented activity in hypothalamic neurons.[64-66]

Pharmacological Evidence

Probably the most compelling evidence for the involvement of CA in LH secretion comes from studies using pharmacological techniques. Intraventricular injection of NE has been shown to induce ovulation[67] and increased LH serum concentrations[58] in rats. Acute blockade of NE synthesis with dopamine-β-hydroxylase (DBH) inhibitors dampens episodic LH release,[68,69] mean serum LH concentrations[70] and the surges of LH induced by electrical stimulation as well as by gonadal steroids.[71,72] Further, the effects of DBH inhibition of LH release could be overcome with dihydroxyphenylserine but not L-DOPA.[72] Similarly, α-adrenergic receptor antagonists blocked ovulation,[73] reduced LH response to castration,[68] and blocked the gonadal steroid induced LH release in ovariectomized rats.[72]

Destruction of the ventral NE pathway by injection of the neurotoxic agent, 6-hydroxydopamine, early on proestrus blocked the LH surge.[74]

Studies designed to identify sites of LHRH-NE interaction microimplanted 6-hydroxydopamine into the POA, SCN and the MBH regions in ovariectomized rats treated with estrogen and progesterone. Implants in the POA and SCN but not the MBH blocked the gonadal steroid induced LH surge and depleted NE in the POA-anterior hypothalamic areas.[75] These results support the concept that NE-LHRH neuronal interactions involve hypothalamic regions rostral to the ME in the ovulatory hypersecretion of LH in rats. Interestingly, after chronic depletion of hypothalamic NE by lesion of the ventral NE bundle, cyclic activity returns after several weeks.[76] These results were interpreted as evidence that NE normally is facilitory for LHRH neuron function, but with prolonged absence of NE influence, the LHRH neurons of young mature animals are able to regain normal function.

Although the majority of studies support a facilitory role for NE in LH secretion, recently an inhibitory role of NE in regulation of LH secretion has been proposed in view of the findings that intraventricular NE infusion[77] and electrical stimulation of the ventral NE bundle[78] can suppress the pulsatile mode of LH secretion in ovariectomized, nonsteroid treated rats. Cáceres and Taleisnik have proposed the inhibitory effects of NE on LH release are mediated through β-adrenergic receptor activation on LHRH neurons, while the facilitory effects involve primarily α-adrenergic receptors.[79] Confirmation of this hypothesis requires further study.

The role of DA in LH regulation is less clearly understood than that of NE in the regulation of LH secretion. Early reports suggested DA exerts a stimulatory influence on LH secretion.[80] However, administration of both DA agonists and antagonists have been reported to inhibit or have no effect on LH secretion while intraventricular injection of DA stimulated or had no effect on LH secretion in the rat (see 49). The observation

that DA antagonists generally have no acute effect on LH release indicates the role of DA is not essential for this phase of LHRH neuron function.[81] Whether DA has a role in the synthesis of LHRH or in modulating the input of other neuronal systems remains to be critically evaluated.

Dopamine-Prolactin Regulation

Dopamine Regulation of Prolactin

The inhibitory effect of DA on prolactin secretion is probably the best characterized neurotransmitter-AP functional relationship. Unlike the other AP hormones, prolactin secretion is normally tonically inhibited by hypothalamic factors. The evidence indicating DA is the primary hypothalamic factor responsible for prolactin inhibition has been reviewed.[49] This evidence includes several studies which indicate DA originating in the TIDA neurons enters the portal vasculature in concentrations which effectively dampen prolactin secretion.[82-84] Further, alterations in TIDA neuron activity and altered DA concentrations in portal blood have been associated with altered states of prolactin secretion.[85,86]

In contrast to the above evidence, several studies suggest DA may not be the sole hypothalamic substance which inhibits prolactin secretion. Purified hypothalamic extracts contained significant prolactin inhibiting activity after all CAs were removed from the extracts.[87-89] Similarly, pretreating hypothalamic-AP coincubation preparations with DA antagonists did not eliminate hypothalamic inhibition of prolactin secretion.[90] *In vivo* studies showed that while injected hypothalamic extracts decreased prolactin secretion induced by suckling, the dose of DA required to inhibit prolactin release was more than 30-fold the amount of DA contained in hypothalamic extracts.[91] Conclusive identification of hypothalamic prolactin inhibiting substances other than DA awaits further study.

Prolactin Regulation of Dopamine

Shortly after the demonstration of DA inhibitory effects on prolactin secretion, Hökfelt and Fuxe showed exogenous prolactin augmented TIDA neuron activity.[92] Studies which extended and confirmed the negative feedback role of prolactin on TIDA neuron activity have been reviewed.[93] More recent studies have also indicated prolactin augments extrahypothalamic DA systems.[94-97] These findings suggest prolactin may exert some of its effects on behavior via CNS pathways outside the hypothalamus. The negative feedback relationship of DA and prolactin is altered by estrogen.[98] The ability of estrogen to augment prolactin secretion[99] appears to result from alterations in the intracellular response of the mammotroph to DA.[100,101] Thus, alterations in portal blood DA levels may not correlate with prolactin secretion in an inverse relationship.

Age-Related Alterations in Reproductive Function

Estrous Cycles in Young Mature Animals

The changing hormone milieu which occurs during the reproductive cycle in young mature females ensures that ova are released under optimal conditions for successful fertilization and implantation. The coordination of cyclic physiological and behavioral changes required for successful reproduction is closely regulated by dynamic fluctuations in hormone secretion and involves interplay of the CNS, AP and ovary. These hormonal interactions have been reviewed.[29,102,103] Briefly, ovulation is triggered by a hypersecretion or surge of LH. The interval between LH surges is characteristic of the length of the ovarian cycle in different species (i.e., 4-5 days in rats and 28 days in women). A surge of FSH is coincident with the LH surge and prolactin is also elevated at this time in rats. Estrogen secretion gradually increases with follicular growth and increases more rapidly prior to the LH surge. Progesterone secretion

is also increased before the LH surge and appears important in inducing estrous behavior in rats (see 103). Progresterone secretion is highest during the luteal phase of the cycle.

The LH surge is triggered by increasing circulating estrogen levels which is the positive feedback effect of estrogen on LH secretion. This effect of estrogen is exerted at both the AP and hypothalamic level since both augmented AP response to LHRH and hypersecretion of LHRH are associated with these positive feedback effects (see 29). In contrast to positive feedback effects of estrogen responsible for the LH surge, tonic basal LH secretion is modulated by the inhibitory feedback actions of estradiol and progesterone. Thus, ovariectomy results in elevated LH levels which occur from secretory bursts or pulses of LH from the AP. Pulsatile LH secretion appears to be the result of pulsatile LHRH release into the portal vasculature (see 29 and 81).

Elucidation of the CNS regulatory mechanisms involved in both positive and negative feedback effects of estradiol used intact cycling animals as well as ovariectomized animals treated with differing regimens of estrogen and/or progesterone (see 103). Results of studies employing these animal models indicate that NE facilitates LHRH neuron secretory activity, while DA neuron activity is more closely associated with prolactin secretion (see 49, 50 and 104).

Patterns of Reproductive Senescence

Although advanced postmaturational age is accompanied by a decline in reproductive function in all species, the patterns of reproductive senescence differ. Patterns of reproductive decline with increasing age have been studied most extensively in women, rats, mice and hamsters. Each of these species shows a decrease in fertility as measured by viable

offspring during the middle third of their normal expected life span (see 105). Accompanying this decrease in fertility is a change from normal, regular ovarian cycles to irregular cycles. Premenopausal women experience shortened-irregular menstrual cycles while rats and mice have lengthened, irregular cycles in middle-age.[106-108] The hamster generally maintains regular estrous cycles through old age (19 months); however, 10-20% of old hamsters have lengthened irregular cycles.[109] The decreased fertility of ovulating middle-aged women and rodents has been associated with altered uterine function (see 105).

Following variable periods of irregular ovarian cycles and diminished fertility, most animals enter states of noncyclic ovarian function or reproductive senescence. In postmenopausal women, ovaries atrophy and become fibrotic. Ovaries from old mice often have well developed follicles which secrete sufficient estrogen to maintain persistent cornified vaginal epithelium.[110] These senescent mice are often classified as "persistent vaginal cornified state" or CE. Other old mice have persistent diestrous vaginal lavages and are acyclic.[111]

Extensive longitudinal studies in Long-Evans and Sprague-Dawley strains of rats revealed the incidence of irregular cycles diminishes and the frequency of CE rats begins to increase between 10 and 12 months of age.[112-114] By 19 months of age most rats experience the CE state. In later life PP animals predominate.[114] Everett characterized the now extinct line of short-lived inbred rats from the Duke Anatomy Department which entered CE between 6 and 8 months of age.[115] In contrast, the long-lived Fisher 344 rat has predominantly irregular cycles in late middle-age and then enters the PP state in late life (20-27 months).[116]

The CE rat has large, well developed follicles, no corpora lutea and moderately elevated levels of serum estrogen compared to diestrous young rats.[117] The old PP rat ovulates at 8 to 20 day intervals and maintains functional corpora lutea between ovulatory events.[117] These ovarian changes are reflected in the vaginal cytology of PP rats which typically have one or two days of cornified epithelial cells intermittent with several consecutive days of predominantly leukocytic smears, while the CE rat shows consistent cornified vaginal epithelium.

The locus of impairment responsible for the diverse patterns of reproductive senescence differs between species. In the mouse and woman ovarian oocyte exhaustion appears primarily responsible for loss of ovarian cyclicity as indicated from two lines of evidence. Hemiovariectomy decreases reproductive life-span by 50% in both mice[118] and women;[119] however, no decrease was observed in hemiovariectomized rats.[120] Conversely, hypophysectomy which retards oocyte loss prolonged ovarian function in mice.[121] When young mice received ovary transplants from old hypophysectomized donor mice, normal cycles resulted.[122] Prior to menopause, ovaries in women have a reduced response to gonadotropin and produce decreased levels of gonadal steroids.[123] This decrease in gonadal steroids may result in shortened menstrual cycles. Postmenopausal women have little or no ovarian response to gonadotropins.

A CNS impairment has been implicated in the noncyclic reproductive states of rats.[124] Indirect evidence includes the results of ovarian transplant studies which showed ovaries from old CE and PP rats regain function upon transplant to young recipients.[125] In contrast to results in human ovaries, gonadotropin receptor responses are not diminished with age in rats.[126] Second, multiple injections of LHRH induced LH secretory

responses in old CE rats which were similar in magnitude to LH response in young animals.[127]

More direct evidence implicating a central nervous system (CNS) dysfunction includes several studies in which successful reinitiation of estrous cycles in old rats has been accomplished with a variety of CNS mediated stimuli (see 105). These stimuli include electrical stimulation of the POA, chronic ether stress, and administration of CNS acting drugs such as dihydroxyphenylalanine (L-DOPA), iproniazid, epinephrine, progesterone and corticotropin.[128-134]

While reproductive decline in aging males is well known, the patterns of alteration are not as well characterized compared to females. Aging is generally associated with decreased libido, decreased sperm production, and declines in function or size of testosterone dependent tissues.[135] The majority of studies indicate that testosterone production generally decreases with age in men and rats (see 135), an apparent result of a decline in the diurnal phase of hypersecretion of testosterone.[136,137] Testicular alterations may be in part responsible for diminished gonadal steroid production.[138] Recent studies which carefully monitored health status of old men and animals suggest that the age-related decline in testosterone may be more closely related to age-related pathologies.[139,140] A central locus for age-related reproductive alterations in men and rats has recently been suggested from studies which examined the diurnal patterns of testosterone secretion in rats[136] and men.[137]

Luteinizing Hormone Secretion

Basal LH secretion changes in different directions in humans compared to laboratory rodents with increasing age. Basal LH secretion in women gradually increases prior to menopause and more rapidly increases after

menopause.[123] Serum LH concentrations during the later decades are similar to levels measured in young ovariectomized women until the ninth decade when LH decreases.[141] Similar LH increases occur with age in men, although the increase is gradual and maintained through the ninth decade.[142] Elevated LH levels appear to result from diminished gonadal steroid secretion since treatment with appropriate gonadal steroids decreases gonadotropin levels to those of young control subjects.[143] In contrast, basal LH secretion in healthy old male laboratory rodents is modestly decreased or unchanged,[144-147], while old female rats[114,148,149] and mice[150,151] have LH levels which are slightly elevated or unchanged compared to young diestrous rodents.

Hypersecretory LH responses appear to be generally reduced with age in laboratory rodents. Several studies have shown the ovulatory surge of LH on proestrus is dampened in middle-aged normal cycling and irregular cycling rats[152-155] and mice.[151] Similarly, the positive feedback LH response to stimulatory regimens of gonadal steroids is impaired in CE rats.[156-159] Interestingly, stimulatory regimens of gonadal steroids were less effective in augmenting LH in postmenopausal women compared to premenopausal women.[160] However, no age-related decreases were shown in similar studies which examined positive LH feedback in hamsters[109] and both normal and decreased[148] LH responses were found in old PP rats. Studies designed to evaluate negative feedback responses consistently showed LH response to castration is slower and lower in aged male and female rats,[144,148,156,158,161-163] female hamsters[109] and male mice.[164] Similarly, the LH response to ether stress was decreased in old male rats.[133]

Both <u>in vivo</u> and <u>in vitro</u> studies suggest that alterations in the pituitary response to LHRH may contribute to dampened hypersecretory LH

responses in old animals. Luteinizing hormone secretion following a single injection of LHRH is reduced in old compared to young male and female rats[127,133,135,162,163,165-167] and female hamsters.[109] Studies using incubated pituitaries from male and female rats showed a large decrease with age in both LH response to LHRH and pituitary LH content.[117,135,168,169] However, more recent studies which monitored AP morphology rigorously found no age-related difference in LH response to LHRH of AP cell cultures between young cycling and old PP rats[170] or *in vivo* LH response of young and old male mice to LHRH.[105] Age-related differences have been shown in the properties of secreted LH both *in vitro*[170] and *in vivo*[171] indicating the biological activity of LH measured with RIA may increase in old rats.

The majority of evidence does not implicate pituitary dysfunction in the initiation or maintenance of noncyclic reproductive states. The magnitude of LH required to stimulate ovulation of graffian follicles is 10 to 20% of the LH hypersecretion measured on proestrus in young rats.[172] Since the degree of decreased LH response to LHRH has never been shown to approach 80%, impaired ability of old pituitaries to secrete LH is unlikely to result in the CE or PP state. Further, multiple injections of LHRH in both male[165] and female CE rats,[127] stimulate LH levels which are comparable to levels assayed in young rats indicating no drastic age-related impairment in LH secretory capacity exists when the pituitary is sufficiently stimulated. The differential diminished response to a single injection of LHRH and near normal response to multiple LHRH injections of old rats suggests that old AP's may require augmented LHRH priming. Alternatively, the diminished response to a single LHRH challenge might result from diminished endogenous LHRH input of old compared to young

rat AP's. The self-priming of LHRH on its own secretion is well described in the rat[173] and appears to participate in the generation of the proestrous LH surge.[29] The latter possibility implies a CNS alteration may change the secretion modes of hypothalamic LHRH input to the AP in old rats.

Prolactin Secretion

The best documented age-related hormone alteration is the increase in basal prolactin levels during advanced age. In man, serum prolactin concentrations are generally stable through the seventh decade and then increase,[174] although prolactin decreases are seen in postmenopausal women as estrogen declines.[174] Elevated prolactin concentrations have been reported consistently in old male and female rats.[113,114,133,144,159,161,175-180] The magnitude and time of age-related increases in prolactin differs with the reproductive status of old rats. Old CE females typically have serum prolactin levels which are more than twice those seen in old male and PP rats.[113,144] Further, prolactin appears to increase during middle-age in strains of rats which predominantly show the CE state with advanced age, while levels are stable through middle-age in strains which enter the PP state late in life.[176] The mouse appears to be exceptional in that serum prolactin levels remain stable throughout the life span[164] or decrease during middle-age.[150,176]

Mechanisms responsible for prolactin increases with age are not clear. Ovariectomy of old CE rats reduces prolactin to levels comparable to young animals[159,161,175] while little postcastration decrease is apparent in PP rats.[161,175] Thus, the elevated estrogen levels associated with the CE state appear to cause prolactin increases in old CE rats. Whether age-related increases in prolactin in PP and male rats result from AP

alterations, diminished hypothalamic inhibition or augmented hypothalamic stimulation of prolactin secretion is unclear. Elevations in human serum prolactin levels during late decades have been associated with a high incidence of pituitary microadenomas compared to young controls.[181] Further, AP adenomas from subjects greater than eighty years of age had positive immunostaining for prolactin, while young hypophysectomized patients predominantly had growth hormone secreting adenomas.[181] The difference in basal prolactin secretion alterations with age in rodents may also be partially attributed to the increased incidence of AP adenomas in rats[182] compared to the low incidence of AP pathology in C57/6J mice.[105] Since the majority of studies which examined AP hormone secretion differences with age have not rigorously monitored health status or AP morphology, the relative contribution of AP pathological conditions to reported age-related differences in neuroendocrine function is difficult to evaluate.

Age-Related Alterations in the Central Nervous System

Dopamine

The well documented increased incidence of movement disorders in older individuals precipitated investigation of age-related alterations in CNS function. Results of these studies have been reviewed.[183] Although the majority of studies examined extrahypothalamic DA systems, the previously discussed changes in AP hormone regulation also stimulated investigation of hypothalamic alterations during advancing age.

In general, there are no massive overall changes of neuronal function in healthy old humans or animals.[105,184] Rather, in the absence of pathological conditions, alterations appear limited to quite focalized brain regions.[105,183,184] Thus, studies which examined whole brain tissues

rarely reported age differences. Studies which do report age changes within brain regions usually report the direction of change is toward impaired DA function. Decreases in DA activity have been reported most consistently in the nigrostriatal DA system. In this region decreased DA concentrations,[185-188] impaired enzymatic synthesis,[189-193] dampened DA turnover[187,188,192,194] and diminished receptor recognition[195-203] have been shown in rodents, rabbits and humans. An initial glance at these alterations suggests their cumulative effects might result in severe functional impairment of this DA system. However, more recent studies indicate the complex mechanisms which regulate neurotransmitter function may at least partially compensate for reported changes. In contrast to several studies which suggest DA binding affinities remain constant while the number of binding sites decrease in striatal and other brain regions (see 204,205), Marquis and coworkers recently reported an increase in the number of striatum-DA binding sites in old mice and rats.[203] These divergent reports may be partially explained by different methods employed to estimate DA binding characteristics since some evidence suggests not all classes of DA receptors are altered with age.[206]

The significance of reported alterations in DA binding sites with age is presently difficult to evaluate. In vitro studies have demonstrated consistently that DA-stimulated adenyl cyclase in the rodent striatum decreases with age.[201,207,208] However, in vivo studies suggest age-related impairments do not involve primarily receptor sites. A series of studies by Joseph and coworkers showed that rotation behavior induced by amphetamines in rats with unilateral lesions of the substantia nigra is attenuated with age.[208-210] However, no age differences were seen in rotation after administering the DA agonist, apomorphine.[208] Further,

treating rats with the DA precursor, L-DOPA, does not restore amphetamine induced rotation deficits in old compared to young lesioned rats.[209] Another study by these investigators showed the extent of DA depletion in substantia nigra after injection of the neurotoxin, 6-hydroxydopamine, was dampened in old compared to young rats.[210] In contrast to these results, two reports from Finch's laboratory did not observe induction of DA receptor proliferation (supersensitivity) in old mice after haloperidol treatment.[202,211] An explanation for these divergent results may lie in species difference or in the methods employed to induce receptor augmentation following drug-induced impairment of DA transmission. Another in vivo approach to examine DA receptor alterations was used by Marshall and Berrios who examined the mechanisms of impaired swimming efficiency in old rats to levels similar to young animals.[212] Collectively, these in vivo data indicate that alterations in the substantia nigra DA system with age appear to primarily involve the ability of neurons to release DA rather than receptor alterations. Anatomical evidence supporting alterations in DA release mechanisms in old mice comes from a study that showed synaptic varicosities associated with stored rather than releasable pools of DA were more prevalent in old mice.[213]

Intrahypothalamic DA systems have not been studied as extensively as the nigrostriatal system. Earlier investigations reported little if any effect of age on DA concentrations in whole hypothalamic tissues.[188,192,214-216] However, examination of DA concentrations within hypothalamic regions revealed age-related variations. Doapmine levels were substantially decreased in the ME of old rats and mice[217-220] and in the MBH of old rats.[215,216] Few studies have examined age-related changes in enzymatic synthesis of hypothalamic DA. Tyrosine hydroxylase activity in hypothalami was increased

in 24 to 26 months old Fischer 344 rats[221] and unchanged in 29 months old Sprague-Dawley rats.[193] Aromatic amino acid decarboxylase was unchanged in the old mouse[221] and decreased in older men.[191]

The activity of hypothalamic DA neurons in old rodents has been examined with two methods. Turnover rates of DA estimated by steady state and nonsteady state methods was decreased in whole hypothalami of male mice[188] and rats[192] and MBH of male rats,[216] but was unchanged in the anterior hypothalamic region of old female Long Evans rats.[216] The latter study saw no augmentation of DA turnover in response to ovariectomy in the anterior hypothalamus which was observed in young rats. However, Finch's laboratory recently reported that turnover was maintained in old male mice ME tissues in spite of a 25% decrease in DA concentration.[218] Another approach examined TIDA neuron activity by measuring DA concentrations in portal blood. These studies found age-related decreases in portal DA blood levels, supporting data which found decreased TIDA turnover during advanced age.[222,223] Histological studies which support decreased TIDA function reported DA was decreased in the ME of old male Fischer 344 rats and increased in NA perikarya[217] and evidence of degraded TIDA neurons was found in old male mice.[224] Collectively, these data indicate that, of the intrahypothalamic DA systems, the TIDA system appears to be more severely affected by increasing age than other DA systems.

Norepinephrine

The majority of studies which examined changes in NE neuron function indicate activity decreases with age, although age-related alterations in NE activity are not massive and also appear to be confined to localized brain regions. Norepinephrine concentrations have been reported to decrease in human and monkey hindbrain and hypothalamic regions[225,226] as well

as in rat hypothalami,[214-216] and MBH tissues.[215,216] The mouse appears exceptional in that NE concentrations remain stable in all regions examined through postmaturational life.[218] However, the rate of NE turnover is decreased in hypothalami from both old mice and rats.[188,192,215] Similarly, the normally observed increase of NE hypothalamic turnover in response to ovariectomy is dampened in old CE rats.[216] Enzyme activity of DBH was shown decreased with age in rats but not in mice.[221] However, a recent study indicates that the augmentation of DBH on proestrus is reduced in old mice.[227]

Alterations in the number or affinity of hypothalamic NE receptors with age have not been characterized. Investigations of extrahypothalamic brain areas indicate both β- and α-adrenergic binding sites decrease in cortex, cerebellum and pineal regions of old rats.[199,228] These regions as well as the hippocampus have decreased adenyl cyclase activity in response of NE stimulation.[208] Further, the proliferation of adrenergic binding sites in response to decreased adrenergic stimulation is dampened in old rats.[229,230] The majority of studies which employed Scatchard analysis suggest that the affinity of binding sites is not altered but rather the number of binding sites diminishes with age.[199,228-230] Although decreases in both α- and β-receptors have been indicated, one study suggests β_1 binding sites may increase with age in the cerebellum of rats.[231] A paucity of information is available on the nature of changes in adrenergic receptors in all brain regions throughout advancing age as most studies compared only young and old rats. The limited availability of tissues from old animals has precluded rigorous examination of binding characteristics within specific brain regions.

Luteinizing Hormone Releasing Hormone

The relationship between altered gonadotropin secretion and LHRH neuron activity in old rats is not clear. Luteinizing hormone releasing hormone concentrations in the hypothalamus of intact female rats were reported to increase,[232] decrease,[220,233,234] or remain stable[235] with age, while LHRH in the MBH of male rats was decreased with age.[217] Explanation for these discrepant results may include the type of assay employed or stage of reproductive senescence of rats examined. Reports employing bioassay methods for LHRH activity found little change with age in whole hypothalamic extracts.[135,169,235] In contrast to studies which found decreased LHRH concentrations with RIA methods, Barnea et al. reported LHRH content increased in synaptosome preparations from whole hypothalami of old rats.[232] These investigators suggested age-related alterations in physiochemical cell properties and subneuronal LHRH distribution may result in impaired ability of old rats to release LHRH. Studies designed to evaluate LHRH neuronal function examined the magnitude of LHRH depletion response following ovariectomy. Wise and Ratner examined this response in young cycling and middle-aged noncycling rats.[234] They found LHRH concentrations were reduced in intact noncycling compared to young rats; however, the extent of LHRH depletion in response to ovariectomy depended on the type of noncyclic reproductive state in older rats. Constant estrous rats had little LHRH depletion response while PP rats had near normal magnitudes of LHRH decrease after ovariectomy.[234] However, Wilkes and Yen reported no diminished LHRH response to ovariectomy in CE rats.[220] These various results indicate the extent of LHRH neuronal alteration with age is not characterized conclusively. Studies which successfully induced ovulation in old noncycling rats (see 105) indicate LHRH

neurons can function sufficiently to release ovulatory quotas of LH. However, the dampened LH surges associated with middle-aged rodents[152-155] may result from mild impairments in LHRH neuron function.

Other Neurosecretory Changes

Age-associated physiological and behavioral impairments have stimulated investigation of other CNS components for alteration during aging in addition to the CA's. Of particular interest to students of neuroendocrine function are the changes in the serotonin system during aging. In man and rhesus monkeys, serotonin was decreased in hypothalamic regions[226,236] but stable in hindbrain areas from postmortem patients.[237] In rodents, hypothalamic concentrations were stable[188,215] or decreased[225] and raphé and hippocampus levels were decreased.[238] Walker et al. have recently suggested that dampened serotonin activity may contribute to impaired cyclic release of LH in old rats.[239] However, changes in the activity of serotonic neurons during age are unclear. Tryptophan hydroxylase has been reported to decrease[238] or remain stable[221] with age in serotonin perikarya of rats. Although the serotonin metabolite, 5-hydroxyindoleacetic acid, has been shown to be increased in both old rat[215] and human[240] tissues, evidence suggests clearance of the metabolite may be decreased with age.[215]

Alterations in cholinergic systems with age have recently been reviewed.[241] Extensive investigation suggests that this system may be impaired in patients who show memory loss; however, the extent of alteration in normal aging is equivocal.[241]

GENERAL RATIONALE

The evidence reviewed in the preceding section indicates that a correlation may exist between changes in central CA metabolism and reproductive dysfunction. These CNS changes may be responsible for decreased LH and elevated prolactin secretion frequently seen in reproductively senescent rats. Two approaches were selected to test this hypothesis. First, studies were undertaken to systemically characterize the pattern of changes in CA metabolism and LHRH levels and to identify the loci of these changes during the postmaturational aging process. Second, this hypothesis was tested directly by examining the effects of pharmacological manipulation of CA systems on AP secretory function in reproductively senescent rats. It is hoped that these approaches will enhance our understanding of the mechanisms which are responsible for sub-optimal neuroendocrine regulation in aging rats. Further, these studies may identify avenues for rational treatment of these neuroendocrine disorders.

GENERAL MATERIALS AND METHODS

Animals

Selection of Animal Model

The laboratory rat was chosen as the experimental animal in the presently described studies for several reasons. First, neuroendocrine interactions between the brain and AP are more thoroughly known for the rat than any other species. Second, the neuroanatomy of both CA and LHRH-containing pathways has been most extensively characterized in the rat. Third, the rat life span is relatively short compared to many other mammals and sufficient numbers of uniformly aged rats could be obtained to investigate neuroendocrine alterations in regulation of LH secretion in this animal model. Additional considerations included the ready access to rat and prolactin RIA systems, size of the animal for surgical manipulation, blood and tissue collection, and the availability of sufficient cages and facilities for long-term housing.

The Long-Evans rat was chosen as the model for studying the CE state because it has a relatively early onset of persistent vaginal cornification,[113,114] appears more resistant to respiratory infection than the Sprague-Dawley rat, and has been the most commonly used animal model to examine age-related alterations in hypothalamic-AP-ovarian regulation. The F344 rat was chosen to examine age-related neuroendocrine changes in the PP state because healthy, barrier-reared old rats of uniform age could be regularly supplied. Additionally, the results of our initial studies showed that the majority of F344 rats (80%) maintain normal estrous cycles until 15 to 18 months of age and then enter the PP state without experiencing the CE state (Table I).

TABLE 1. Reproductive Status of Fischer 344 Rats at Various Ages

	Age (months)		
	9-12	13-20	21-27
Number of Animals	101	44	53
Reproductive Status		Percentage[a]	
Normally Cycling (NC)	81	30	6
Irregularly Cycling (IC)	18	16	6
Constant Estrous (CE)	1	5	0
Repeated Pseudopregnant (PP)	0	50	88

[a]Nearly 100% of animals less than nine months of age showed normal four to five day estrous cycles. All animals included in this Table were monitored for at least 30 days.

Establishment of Rat Colony

Studies which employ aged animals are subject to some considerations which are usually not aspects of experiments that use only young mature animals. Of primary consideration is the source of old animals. Animals for most of these studies came from two sources. Barrier-reared Fischer 344 rats were purchased at 2-3, 9-10 and 19-21 months of age from Charles Rivers Laboratories (Wilmington, MA) through an arrangement with the National Institute on Aging. Because old Long-Evans rats are not commercially available, 150 Long-Evans retired breeder females were purchased from Blue Spruce Farms (Altamont, NY) at 8-10 months of age and 50 to 75 animals at 8-10 months of age were purchased at 3-4 month intervals thereafter. This "stocking" purchase scheme of retired breeders combined with purchase of 2-3 month old animals resulted in sufficient numbers of animals of the same cohort and reproductive status to ensure completion of these experiments.

Animals were housed two per cage in one of three animal rooms upon arrival at the animal facility. Rats were provided free access to Purina Rat Chow (Ralston Purina Co., St. Louis, MO) and tap water. Rooms are temperature (23° ± 2°C) and light (lights on 0500 to 1900 h) controlled and each room houses animals from a single source. In order to minimize the incidence of murine pneumonia in the colony, two precautions were taken. First, animal rooms were kept locked to minimize traffic. Second, animal technicians fed, watered and cleaned the senescent colony at the beginning of their work day prior to entering other animal rooms. Only one episode of respiratory distress arose in the colony in three years. In this instance, tetracycline was added to the drinking water for a seven day period and thereafter animals showed no signs of pneumonia.

Monitoring Cycle Status

Animal reproductive status was monitored by histological examination of vaginal lavages.[108] Each animal was "smeared" for ten consecutive days each month and classified for that month according to the following criteria:

-- Normally cycling (NC) rats - lavages showed two estrous cycles each, four to five days in length;

-- Constant estrus (CE) rats - lavages showed ten consecutive days with cornified epithelium;

-- Repeated pseudopregnant (PP) rats - lavages showed ten consecutive days of predominant leukocytes or one or two days of cornified epithelium;

-- Irregular cycling (IC) rats - lavages showed several consecutive days of cornified epithelium or several days of leukocytes.

One month prior to the experimental day, animals were selected on the basis of cycle history and assigned to treatment groups. Vaginal lavages were then examined daily through the end of the experimental period. This monitoring procedure ensured animals with similar reproductive histories could be assigned to experimental groups. Although the ten day evaluation period alone may not have been sufficient to always distinguish PP and IC rats, constant cycle evaluation is extremely tedious, time consuming and costly. Daily monitoring of animals assigned to treatment groups 30 days prior to the experimental period ensured reproductive status was accurately evaluated.

Monitoring Health Status

Separating normal age-related physiological alterations from pathological conditions that occur with increased frequency in advanced age is

a major consideration in gerontological studies.[242] Three routine procedures were employed to minimize the contributions of possible pathological effects to experimental results in these experiments. First, general animal robustness was monitored during the period of reproductive cycle evaluation. Daily handling ensured that animals with observable tumors, skin lesions, abnormal urinary tract discharges, weight loss or behavior alterations were identified and could be removed from the colony. Second, animals were necropsied upon completion of the experiment for visible abnormalities. Viscera were examined for gross pathology and kidney, heart, adrenal, and pituitary weights were recorded. Although this procedure could not detect many pathological conditions, extensive histological necropsy of each animal was not feasible. Third, because the incidence of prolactin secreting adenomas is relatively high in Long-Evans old rats,[180,182] AP tissues were assayed for prolactin and LH content. Animals with disproportionately high ratios of prolactin versus LH were omitted from the experiments.

The monitoring procedure 30 days prior to the experiment and at the termination of the experiment routinely results in elimination of some old animals from each treatment group. Therefore, group sizes were augmented 10 to 20% at the time old animals were assigned to treatment groups.

Surgical Treatment and Blood Collection

Animals in experiments using castrated rats were ovariectomized under deep ether anesthesia two weeks prior to the experimental day. A bilateral dorsal surgical approach was used for ovariectomies. Following surgery, animals were monitored for wound healing.

Blood was obtained by one of two methods. Several experiments examined trunk blood collected at decapitation. Decapitations were completed within

30 seconds of removal of each rat from its home cage. Since decapitation experiments were designed to characterize brain CA and LHRH neuron function, trunk blood was collected while brains were rapidly exteriorized and frozen on dry ice. These procedures were completed within 30 seconds of decapitation. Posterior pituitaries were dissected and homogenized in 40 µl of 0.4 N perchloric acid for CA assay. Anterior pituitaries were dissected, weighed and homogenized in phosphate buffered saline for later LH and prolactin assay. Animals were then necropsied as described above.

Experiments designed to characterize the pulsatile nature of hormone secretion employed cannulated rats. Silastic catheters (id. 0.025 in, od 0.047 in, Dow Corning, Midland, MI) were implanted into the atrium via the right jugular vein[243] while rats were under deep anesthesia induced by chloral hydrate (400 mg/Kg body weight, ip) or pentobarbital (40 mg/Kg body weight, sc). Catheters were exteriorized on the dorsal aspect of the neck, filled with heparin (1000 units/ml) and stoppered with stainless steel plugs. Animals were then individually housed. The following day, silastic extension tubes (30 cm long, id 0.025 in, od 0.047 in) were connected to the catheters and draped outside of the animal's home cage. One milliliter syringes connected to the extension tubes permitted repeated blood sampling from freely moving animals. The syringe weight prevented the extension tubes from tangling without hindering rat movement. Thirty or 60 min prior to blood sampling, rats received 200 units of heparin (200 µl) via the catheter.

Serial blood samples were then obtained at 10 or 15 min intervals for a 3 h period. Saline filling the cathether (about 100 µl) was removed and discarded. For studies which examined LH profiles at 15 min intervals,

300 µl of blood was removed for assay of plasma LH and an equal volume of 0.9% saline was returned to the animal via the catheter. During the 3 h period, hematocrits decreased 10 to 25%. In studies which examined LH fluctuations in samples collected at 10 min intervals, 500 µl of blood was removed via the catheter. Blood samples were centrifuged (Beckman Microfuge B, Palo Alto, CA) for 30 sec, and 200 µl of plasma was separated for assay of LH. Cells were resuspended in 200 µl of heparinized saline (5 units/ml) and returned to the same animal following the next blood sample. This procedure resulted in a 0 to 10% decrease in the hematocrit during the 3 h sampling period. Animals were then necropsied as described above.

Hormone Radioimmunoassays

Plasma and serum samples were assayed for LH and prolactin in duplicate using routine RIA methods described in assay kits provided by the National Institutes of Arthritic, Metabolic and Digestive Diseases (NIAMDD). Pituitaries were homogenized and diluted in phosphate buffered saline containing 1% gelatin. Diluted homogenates were assayed for hormone levels in quadruplicate. Hormone concentrations are expressed in terms of the standard reference preparations NIAMDD rat LH-RP-1 for LH and NIAMDD rat PRL-RP-1 or PRL-RP-2 for prolactin. These reference standards have respective biological potencies of 0.03 x NIH-LH-S1 (ovarian ascorbic acid depletion test), 11 international units of prolactin (mouse deciduoma test) and 30 international units of prolactin (pigeon local crop sac assay). Minimum assay sensitivities were 1 ng for LH and 0.05 ng for prolactin per assay tube.

Levels of LHRH in supernatants of hypothalamic tissues homogenized in 0.1 N hydrochloric acid were determined using previously described RIA

methods.[244] Acid supernatants were neutralized with 0.1 N sodium hydroxide during the assay procedure. Synthetic LHRH obtained from Beckman Co. (Palo Alto, CA) was used as reference standard and for radioiodination. Monoiodo-LHRH was employed in the assay.[245] Rabbit antibodies against LHRH were generously provided by Dr. Nett (R-42, Colorado State University, Fort Collins, CO) or purchased from Miles Laboratories (Elkhart, IN). Minimum LHRH assay sensitivity was established as 2 pg/tube at which 10% of total labelled binding was displaced for both LHRH antibodies. Concentrations of LHRH were expressed in terms of protein measured with the dye binding method of Bradford[246] in pellets formed after centrifuging tissue homogenates.

Samples from individual experiments were evaluated in a single assay to avoid interassay variability. Hormone concentrations were determined only from serum or homogenate assay volumes which resulted in values on the linear portion of the standard curves.

Microdissection of Brain Areas

Five experiments designed to examine regional brain CA and LHRH alterations with age utilized the microdissection method first described by Palkovits.[24] This "punch" technique has been used extensively to map the distribution of neurotransmitters,[24,35,247] neuropeptides,[24,50,248] and neurotransmitter synthetic enzymes[63,249] within the rat brain.

Brains which were collected during the morning were transferred from dry ice to a cryostat chamber (IEC model CTD-Harris Cryostat, Needham Heights, MA) held at knife temperature of -10°C. Serial frontal sections (300 μm thick) were then cut beginning rostral to the NAc or OVLT and extended through the mammillary bodies. Regions were identified in frozen sections under a stereomicroscope with the aid of König and Klippel's

TABLE II. Parameters Used in Microdissection of Brain Regions

Region Dissected[a]	Approximate Coordinate[b]	Punches/ Brain[c]	Needles I.D. (mm)
NAc	A 9410	4	1.0
OVLT	A 7770	1-2	0.5
Striatum	A 7470	4	1.0
POAm	A 7200	4	1.0
POAs	A 7020	4	0.75
NHA	A 6360	4	1.0
MFB	A 6360	2	0.75
NSO	A 6360	2	0.75
NSC	A 6060	2	0.75
ARC	A 5660	2	0.75
NVM	A 4950	6	0.75
NA	A 4950	6-8	0.5
ME	A 4950	6-8	0.5

[a]Abbreviations used are as follows: nucleus accumbens (NAc); organum vasculosum of the lamini terminalis (OVLT); preoptic area medialis (POAm); preoptic area suprachiasmatica (POAs); anterior hypothalamic nucleus (NHA); medial forebrain bundle (MFB); nucleus supraoptica (NSO); nucleus suprachiasmatica (NSC); area retrochiasmatica (ARC); nucleus ventromedialis (NVM); nucleus arcuate (NA); median eminence (ME).

[b]The coordinates listed utilized the orientation of König and Klippel[250] and represent the first slice from which nuclei were punched. For several regions punches were taken from subsequent slices and pooled.

[c]Except for the OVLT, regions were dissected bilaterally. For the ME, overlapping bilateral punches were taken to include the DA rich lateral palisades zones.[251]

stereotaxic atlas.[250] Table II presents details of the microdissection parameters employed in these studies. Microdissected tissues were immediately homogenized in 40 µl of 0.1 N perchloric acid containing 10 mg EDTA/100 ml and frozen for later CA assay. Microdissection was completed with 8 h of decapitation and acid homogenates were stored at -20°C or -80°C until assay.

The internal diameter of needles used for microdissection in these studies was 1.5 to 2.5 times the diameter of punches used in the original description of this technique.[24] Previous studies reported the need to pool tissue obtained from two to three animals to estimate CA concentrations in some hypothalamic regions.[24] The present studies anticipated lower CA levels in old animals and animals treated with α-methyl paratyrosine (αMPT) compared to these other reports. Since the high cost and limited availability of old rats precluded the option of pooling tissues from several animals, larger punch sizes were examined. The internal diameter of needles employed in these studies dissected the entire region of interest and in several areas, some surrounding tissue. All regions except the OVLT were dissected bilaterally. The ME region dissected contained only anatomically distinct ME tissue.[251]

Hypothalamic nuclei examined for LHRH and CA concentrations in these studies were selected on the basis of their suspected roles in the regulation of LH secretion[24,50] or because they were terminal beds for various CA neuronal systems.

Determination of Catecholamine Neuron Activity

Although altered CA concentrations during various physiological or experimentally induced states suggest that functional changes in the release of neurotransmitters might be present, this approach does not resolve

whether concentration differences result from altered release or synthesis. Additionally, several reports have shown that CA concentrations remain remarkably stable in spite of large differences in neuron activity.[252] Because direct measurements of CA release from presynaptic terminals is presently not feasible, several approaches have been used to estimate the neuronal activity of CA systems. Each method used thus far has some limitations which have been reviewed.[252]

The present studies used the nonsteady state method of Brodie et al.[253] to estimate CA activity within microdissected brain regions. This method measures the rate of CA depletion following blockade of CA synthesis with the tyrosine hydroxylase inhibitor, αMPT. It assumes that the rate of CA depletion is equivalent to the rate of CA release. An advantage of this method over other nonsteady state methods which measure accumulation of DA, NE or their metabolites lies in evidence that feedback inhibition of these accumulated products may decrease the synthesis of CA's (see 50). It has also been shown that αMPT treatment does not alter the uptake or storage capacity of CA neurons.[254]

Steady state methods to monitor activity of CA neurons inject trace doses of labelled CA or precursors which do not disturb the steady state CA levels. The rate of accumulated labelled CA after injection of precursors or the rate of labelled CA disappearance after injection of radioactive amines reflects neuron activity. These methods assume a uniform distribution of injected trace material into the CA neuronal pool and further assume that the administered amounts do not disturb the ongoing neuron activity. While these assumptions are subject to some controversy[252] the major disadvantage of this approach is the large amount of tissue required to measure activities.

The major advantage of the αMPT method lies in the ability to detect changes in CA neuron activity within small tissue regions. Two cautions must be kept in mind using this approach. First, since the rate of CA depletion after synthesis inhibition exhibits first order kinetics,[253] the times after drug treatment chosen to examine CA concentrations should be selected over periods that have detectable CA levels. Second, the method assumes that indirect effects of αMPT on hormone secretion (such as the increase in prolactin with DA decreases) do not result in feedback alterations on activity of CA neurons. It should also be stressed that this method reflects relative neuron activities which should be compared to different experimental groups examined under the same conditions. Thus, the direction of changes have been shown to be similar in physiological states tested under different experimental conditions, but the values reported for CA activity vary among experiments.

The experimental procedure for estimating CA turnover by this non-steady state method is as follows. On the morning of experiment, 30 animals from each age group were divided into three subgroups. Two of the subgroups were treated with αMPT (250 mg/kg ip, Sigma Chemical Co., St. Louis, MO) and were killed by decapitation either 30 and 60 or 45 and 90 min later. As a control for possible stress-related effects of ip injection, the third subgroup received saline in place of αMPT and was killed 30 or 45 min after injection. Tissues were then collected and processed as previously described.

Turnover rates of NE and DA (ng/mg protein/h) were calculated for each brain region from the product of the rate constant of amine loss and the steady state CA concentration (ng/mg protein) as described by Brodie et al.[253] The rate constant was determined from least squares fit analysis of the αMPT induced depletion for DA and NE within each area. The rate constant

of amine loss reflects the activity of CA neurons while the turnover rates indicate the relative amounts of CA released for postsynaptic recognition in the tissue region examined.[253] Age-related differences in turnover rates were tested by LSD tests after determining the variance of the turnover rate from application of the Taylor expansion formula.[255] Since treatment with αMPT reduced CA levels to below the detectable limits of the assay in some regions, turnover rates were determined only for regions which had measurable CA levels throughout the experimental period.

Catecholamine Radioenzymatic Assay

Acid homogenates of microdissected tissues were thawed and centrifuged (Beckman Microfuge B, Palo Alto, CA) for 90 sec immediately prior to evaluation for DA and NE content. Supernatant samples were assayed in volumes of 5 or 10 μl for DA and NE using a modification of the radioenzymatic method of Cuello et al.[256] as previously described.[42] This method employs catechol-O-methyl transferase, isolated from rat livers,[257] to catalyze the O-methylation with tritiated S-adenosyl methionine (New England Nuclear, Boston, MA) of DA and NE to methoxytyramine and normetanephrine, respectively. Tritiated metabolites were separated by thin layer chromatography, identified under ultraviolet light and fluorescent spots were scraped for quantification of tritiated activity with liquid scintillation.[258] The separation procedures employed resulted in complete separation of the NE metabolite and less than 2% normetanephrine contamination in the DA metabolite fraction.[42]

Each microdissected region was evaluated in a separate assay to eliminate interassay variability within a region. Minimum assay sensitivities, calculated from twice the tritium activity obtained from blank standards, were approximately 20 pg for DA and 50 pg for NE. Protein content of the

pellet obtained after centrifugation was measured with the dye binding method of Bradford.[246] Bovine serum albumin was the protein standard and CA values are expressed in terms of protein concentrations.

EXPERIMENTAL

Evaluation of Age-Related Alterations in Catecholamine and Luteinizing Hormone Releasing Hormone Neuronal Activity Within Microdissected Brain Regions

Changes in Catecholamine Concentrations in Microdissected Brain Regions of Aged Male Rats

Objectives. A large body of evidence has implicated a role for central CA neurons in the secretion of LH and prolactin from the AP of the rat.[25,49,50,104] Although the distribution of DA and NE among discrete regions of the brains of young mature animals has been well characterized,[24] little is known about the distribution of these putative neurotransmitters in the aged rat. Since the secretion of LH and prolactin appear to be dependent upon CA activity in discrete regions along the preopticotuberal pathway and hormone secretory patterns are altered in old rats (see 113, 148, 182), we compared the concentrations of DA and NE in discrete regions of the brain structures in young and old male rats.

This study was undertaken to establish both the feasibility of measuring CA from microdissected brain regions in our laboratory and to demonstrate that differential CA alterations occur between neural regions of aged rats. Male rats were initially used in this study because they do not have variation in hormone levels and CA fluctuation due to cyclic changes in ovarian cycles.

Materials and Methods. Male Wistar rats (Harlan Industries, Indianapolis, IN) 3-4 months (n=8) and 24-25 months (n=7) of age were housed in our animal facilities for one week. On the morning of experiment animals were decapitated, brains were removed and frozen on dry ice and trunk blood

was collected for later assay of serum hormones. Within 2 h following decapitation, 11 POA and hypothalamic nuclear regions were microdissected, homogenized in perchloric acid (containing EDTA 10/mg/100 ml) and frozen for CA assay as described above. The CA assay was sensitive to 31 pg of DA and 30 pg of NE.

Results. Table III presents the changes detected in CA concentration with age for each region examined. Abbreviations used for each dissected region are shown on Table II. Concentrations of NE were significantly decreased in the NA, POAm and OVLT of aged, compared to young, mature male rats. As anticipated, DA concentrations were decreased significantly in some areas. Concentrations of DA were decreased nearly 50% in the ME, ARC and NA. In contrast, DA concentrations were increased 35% in the NHA, 290% in the POAm and 220% in the POAs of old versus young rats.

Mean serum prolactin concentrations were increased significantly in the 23-24 month old rats (165 ± 47 ng/ml) compared to 3-4 month old males (37 ± 4 ng/ml), while serum LH concentrations were not different in young (14 ± 4 ng/ml) and old (16 ± 8 ng/ml) rats. It was noted that three of seven aged rats had AP adenomas which were identified from weights greater than 30 mg. However, neither brain CA concentrations nor serum hormone levels differed consistently among old tumor and nontumor bearing rats.

Discussion. These results demonstrate that differential alterations are present and can be detected with methods employed in our laboratory in CA concentrations among microdissected POA and hypothalamic regions of old rats. The decreased DA in the MBH region and elevated serum prolactin measured in old rats indicates that mammotrophs of old animals may augment prolactin secretion as a result of diminished activity of the TIDA system. The somewhat surprising increases of DA measured in POA regions from old

TABLE III. Age-Related Alterations in the Dopamine (DA) and Norepinephrine (NE) Concentrations in Microdissected Brain Regions from Male Rats

Area	DA (ng/mg Protein) Age (Months)		NE (ng/mg Protein) Age (Months)	
	3-4	24-25	3-4	24-25
ME	69.4 ± 9.8[a]	37.0 ± 4.3**	30.8 ± 5.8	21.2 ± 2.6
NA	15.2 ± 2.5	9.7 ± 1.2*	19.0 ± 1.7	15.3 ± 1.0*
ARC	4.1 ± 0.5	2.1 ± 0.4***	35.7 ± 3.6	29.0 ± 4.9
NVM	3.9 ± 0.5	3.7 ± 0.9	28.6 ± 1.4	21.4 ± 5.1
NSC	2.3 ± 0.3	2.1 ± 0.5	25.7 ± 1.9	27.4 ± 3.2
NSO	2.7 ± 0.6	2.8 ± 1.5	18.8 ± 1.9	17.4 ± 1.4
MFB	4.8 ± 1.1	2.6 ± 0.5	14.8 ± 1.7	14.9 ± 2.1
NHA	2.3 ± 0.3	3.1 ± 0.3*	22.5 ± 1.3	24.7 ± 1.6
POAm	14.1 ± 5.9	55.2 ± 18.0**	72.3 ± 6.0	52.8 ± 7.1*
POAs	3.9 ± 0.5	12.4 ± 4.3*	30.8 ± 2.9	22.5 ± 3.7
OVLT	28.0 ± 6.2	20.2 ± 8.3	35.3 ± 6.9	18.3 ± 3.4*

[a]means ± SEM; * = $p < 0.1$; ** = $p < 0.05$; *** = $p < 0.01$

rats further suggests that DA neuronal systems may respond different to the effects of advanced age.

While NE concentrations were significantly decreased in several POA and hypothalamic regions, basal LH concentrations were not different in young and old male rats. Thus, NE neuronal function in old male rats appears to be adequate to maintain basal secretion of LH. However, old male and female rats are less able to secrete LH in response to several centrally mediated stimuli.[133,148,156-159,161,162] This may indicate that a functional deficiency in NE may become apparent when challenged to augment LH secretion.

Therefore, these results clearly point out the need to examine more closely the activities of CA neurons within these hypothalamic regions and to characterize the extent of alterations in these systems during advanced age.

Changes in Catecholamine Activity in Microdissected Brain Regions of Aging Ovariectomized Fischer 344 Rats

Objectives. Several patterns of reproductive senescence have been described in the female rat.[113,114] While the ovaries and pituitary of the old CE rat can be induced to function relatively normally (see 105), a hypothalamic deficiency appears to prevent the steroid-induced cyclic release of LH in these old animals.[182] This deficiency appears to involve CA neurons, since hypothalamic NE concentrations have been reported to be decreased in old ovariectomized CE rats[113] and several drugs which enhance CA activities can reinitiate estrous cycles in animals experiencing this reproductive state (see 105).

The PP state occurs late in life in a small proportion of Long-Evans and Sprague-Dawley rats[114] and in a high proportion of F344 rats[116] (Table I). In the PP state, animals ovulate at 8 to 20 day intervals with maintained

functional corpora lutea between ovulatory events.[113] The LH secretory capacity of PP rats appears to be relatively normal in response to a stimulatory regimen of gonadal steroids[162] which is in contrast to the response reported in old CE rats.[156-159,162]

In view of the paucity of information on regional alterations in brain CA metabolism during reproductive senescence, this study was undertaken to determine NE and DA concentrations and neuron activity in aging F344 rats which enter the PP state in advanced age. Regions selected for analysis were primarily those along the preopticotuberal pathway, and also regions which contain extensive DA nerve terminal fields from several DA systems. Animals were examined at two ages prior to the PP state and after establishment of the PP state. Animals were examined two weeks after ovariectomy. This experimental design was employed to eliminate the confounding effects of cyclic variation in gonadal hormones on CA activity. Although experimentally induced pseudopregnancy may have served as a control in young animals, it is unclear whether similar mechanisms control the PP state of old rats. Further, the ovariectomized rat model has the advantage of examining CA neuron activity during a state in which hormone response differences have been demonstrated to occur with age.[144,148,156,161-163]

Materials and Methods. Barrier-reared F344 female rats were employed in these studies. Animals were purchased at 3-4, 8-9 and 20-21 months of age and housed in our animal facility. After 30 days of daily monitoring of vaginal cytology, NC young (4-5 months) and middle-aged (9-10 months) and PP old (21-22 months) old rats were ovariectomized. Two weeks later, 30 animals of each age group were divided into three subgroups. Rats in the two subgroups receiving αMPT were killed 45 and 90 min following drug administration while the third saline subgroup was killed 45 min after

TABLE IV. Body and Organ Weights and Health Status of Fischer 344 Rats Employed in Catecholamine Studies

		Age (Months)		
		4-5	9-10	21-22
Number of Rats		30	29	27
Body Weight (g, B.W.)		210 ± 2.4	218 ± 2.5	294 ± 5.7
Anterior Pituitary	Weight (mg)	9.85 ± 0.19	10.10 ± 0.20	15.1 ± 3.4
	mg/100 g B.W.	4.7 ± 0.1	4.6 ± 0.1	3.9 ± 0.2
Adrenal	Weight (mg)	45.6 ± 0.8	44.4 ± 0.9	52.9 ± 1.4
	mg/100 g B.W.	21.7 ± 0.4	20.5 ± 0.5	18.1 ± 0.5
Kidney	Weight (g)	1.57 ± 0.03	1.70 ± 0.03	2.29 ± 0.06
	mg/100 g B.W.	745 ± 14	780 ± 13	784 ± 21
Heart	Weight (mg)	505 ± 7	527 ± 6	730 ± 11
	mg/100 g B.W.	240 ± 2	245 ± 2	240 ± 4
Abnormalities		Number per group		
Pituitary		0	0	6
Lung		4	3	4
Liver		1	1	3

injection. Tissues were collected as described above and animals were necropsied. Minimum sensitivity of the CA assays was approximately 20 pg for DA and 50 pg for NE.

Results. The evaluation of health status of the animals employed in this experiment is shown in Table IV. Both body and organ weights increased with age. When organ weights were expressed as proportions of body weight, no age-related changes were apparent. The incidence of pituitary and liver abnormalities increased in old animals. However, these lesions involved only a small portion of the organ and did not affect organ weight (Table IV). Further, no consistent trends in hormone or CA levels were apparent between lesioned and nonlesioned animals.

Steady state DA concentrations which were measured in every region are shown in Table V for the three age groups. Concentrations of DA decreased in old versus young rats in several areas. These decreases were most dramatic (42 to 78%) in the POAs, ARC, ME and NIL. In the NSC, DA concentrations were reduced 78% to levels below the limits of sensitivity of the assay in the old age group.

Age-related changes in DA turnover rates are shown in Figure 1 for those areas in which DA was still detectable after αMPT treatment. In contrast to the general decreases found in DA concentrations, DA turnover rates were stable, decreased or increased with age in various regions. Turnover rates of DA decreased significantly in the OVLT (81%), POAs (49%) and NA (63%) of old compared with young rats. In contrast, DA turnover increased significantly by 100% in the NVM, 42% in the ME and 22% in the striatum. No significant alteration in DA turnover with age was detected in the POAm or the NIL.

TABLE V. Age-Related Change in Dopamine Activity Within Microdissected Brain Regions of Ovariectomized Fischer 344 Rats

Area	Age (Months)	Dopamine Concentrations (ng/mg protein)	Rate Constant (K)	Turnover Rate (ng/mg protein/hr)
OVLT	4-5	16.7 ± 1.8	0.37 ± 0.07	6.2 ± 0.4
	9-10	13.3 ± 0.7	0.07 ± 0.03*	0.9 ± 0.1*
	21-22	13.7 ± 1.2	0.09 ± 0.05*	1.2 ± 0.1*
S	4-5	61.0 ± 7.1	0.26 ± 0.06	16.1 ± 1.3
	9-10	54.2 ± 6.6	0.26 ± 0.07	14.0 ± 1.3
	21-22	54.3 ± 6.4	0.36 ± 0.08	19.6 ± 1.6†
POAm	4-5	2.5 ± 0.5	0.43 ± 0.07	1.1 ± 0.1
	9-10	3.6 ± 1.0	0.48 ± 0.13	1.7 ± 0.3
	21-22	2.4 ± 0.3	0.60 ± 0.11	1.4 ± 0.1
POAs	4-5	4.0 ± 0.3	0.77 ± 0.07	3.1 ± 0.1
	9-10	3.3 ± 0.6	0.53 ± 0.10	1.7 ± 0.2*
	21-22	2.1 ± 0.2*	0.75 ± 0.09	1.6 ± 0.1*
NA	4-5	27.5 ± 2.0	0.35 ± 0.06	9.6 ± 0.5
	9-10	25.9 ± 5.5	0.31 ± 0.11	8.1 ± 1.1
	21-22	19.6 ± 4.2	0.18 ± 0.11	3.5 ± 0.6*†
NVM	4-5	1.5 ± 0.3	0.49 ± 0.10	0.7 ± 0.1
	9-10	2.3 ± 0.2	0.38 ± 0.10	0.9 ± 0.1
	21-22	1.9 ± 0.2	0.77 ± 0.07†	1.5 ± 0.1*†
ME	4-5	98.7 ± 10.3	0.68 ± 0.09	67.5 ± 4.4
	9-10	109.2 ± 2.0	0.73 ± 0.12	79.3 ± 2.6
	21-22	56.8 ± 11.8*†	1.67 ± 0.17*†	95.1 ± 12.5*
NIL	4-5	6.5 ± 0.8	0.46 ± 0.06	3.0 ± 0.2
	9-10	6.3 ± 0.6	0.55 ± 0.08	3.5 ± 0.2
	21-22	3.4 ± 0.3*†	0.84 ± 0.08*†	2.9 ± 0.2
MFB	4-5	3.0 ± 0.5	ND	ND
	9-10	2.8 ± 0.5	ND	ND
	21-22	1.8 ± 0.8	ND	ND
NSC	4-5	2.2 ± 1.2	ND	ND
	9-10	3.2 ± 0.7	ND	ND
	21-22	ND	ND	ND
ARC	4-5	4.4 ± 0.9	ND	ND
	9-10	2.1 ± 0.3	ND	ND
	21-22	1.3 ± 0.3	ND	ND
NHA	4-5	1.4 ± 0.4	ND	ND
	9-10	1.9 ± 0.3	ND	ND
	21-22	1.2 ± 0.3	ND	ND

*$p < 0.05$ vs 4-5 mo; † $p < 0.05$ vs 9-10 mo.; ND = Rate Constant and Turnover Rate could not be accurately determined (see text)

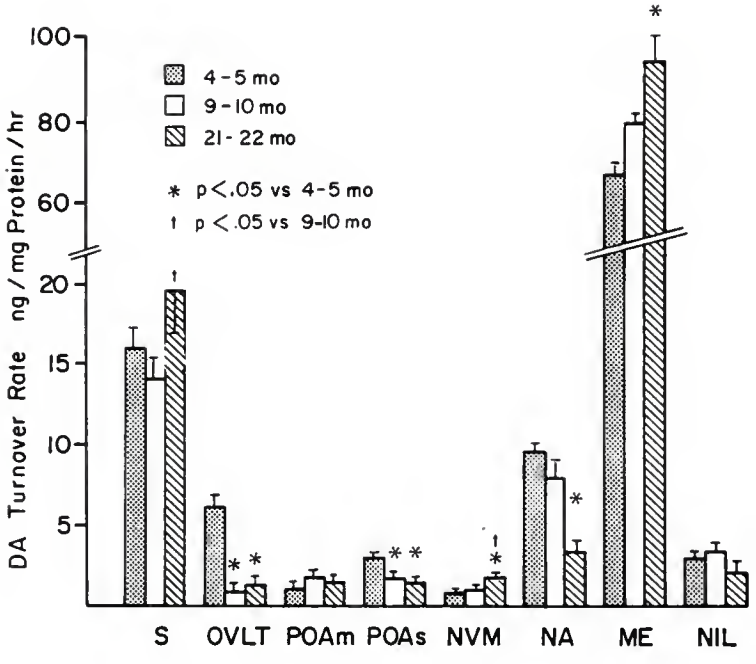

Figure 1. Age-Related Changes in Dopamine (DA) Turnover Rates in Microdissected Brain Regions of Ovariectomized Fischer 344 Rats. Each column represents mean turnover rate as determined by a non-steady state method employing 24-30 rats. Only those regions for which turnover rats could be accurately determined are included. Abbreviations used are the same as in Table II.

The decline in DA turnover rates with age in the OVLT appears to be a consequence of a decreased activity in DA neurons since, while DA concentrations were not changed, the K of DA loss was substantially decreased. The acceleration in the DA turnover observed in the NVM and the ME of old rats was the result of a substantial increase in the K of DA loss. Interestingly, in both the NIL and ME the activity of DA neurons increased coincident with a 48 and 42% decline, respectively, in DA concentrations in these two brain regions.

Table VI illustrates differences in steady state NE concentrations between age groups for each area examined. As found for DA, NE concentrations were decreased in old compared to younger animals in several areas. Relatively large decreases in NE concentration between 4-5 and 21-22 month old animals were observed in the POAs (56%), ARC (56%), MFB (48%), NHA (46%), NA (42%) and NSC (38%). No significant differences in NE concentration were detected between young and middle-aged rats in any area examined.

Turnover rate changes of NE with age present a strikingly different picture from the generalized decreases in NE concentration detected in old rats. As shown in Figure 2, significant increases in NE turnover rates were found in middle age compared to 4-5 month old rats in the POAm (31%), NHA (3.0-fold), MFB (2.9-fold), NSC (2.6-fold) and NA (11.6-fold) regions. Interestingly, NE steady state concentrations did not change with age in the ME but the NE turnover rate increased 5-fold in old animals in this region (Table VI and Figure 2). With the exception of the POAs, the pattern which emerges from these data suggests that increases in NE turnover rates precede the decreases in NE concentrations within discrete hypothalamic areas during the aging process. The increase in turnover rates observed in several regions in middle-aged rats was due primarily to increased K of

TABLE VI. Age-Related Change in Norepinephrine Activity Within Microdissected Brain Regions of Ovariectomized Fischer 344 Rats

Area	Age (months)	Norepinephrine Concentrations (ng/mg protein)	Rate Constant (K)	Turnover Rate (ng/mg protein/hr)
POAm	4-5	10.6 ± 1.6	0.24 ± 0.05	2.5 ± 0.2
	9-10	11.0 ± 1.4	0.33 ± 0.08	3.6 ± 0.3*
	21-22	8.5 ± 1.4	0.32 ± 0.07	2.7 ± 0.3†
POAs	4-5	25.2 ± 2.4	0.35 ± 0.06	8.9 ± 0.6
	9-10	20.2 ± 3.2	0.28 ± 0.10	5.7 ± 0.6*
	21-22	11.1 ± 1.1*†	0.53 ± 0.09†	5.9 ± 0.4*†
MFB	4-5	11.6 ± 1.0	0.30 ± 0.09	3.5 ± 0.3
	9-10	14.6 ± 1.9	0.69 ± 0.08*	10.2 ± 0.8*
	21-22	6.0 ± 0.8*†	0.19 ± 0.15	1.1 ± 0.2*†
NHA	4-5	12.0 ± 3.9	0.29 ± 0.17	3.5 ± 0.7
	9-10	13.6 ± 2.6	0.72 ± 0.13	10.3 ± 1.2*
	21-22	6.5 ± 1.3	0.42 ± 0.21	2.7 ± 0.4†
NSC	4-5	10.3 ± 2.9	0.29 ± 0.10	3.0 ± 0.5
	9-10	9.6 ± 1.7	0.80 ± 0.09*	7.7 ± 0.8*
	21-22	6.4 ± 0.6	0.49 ± 0.09†	3.1 ± 0.2†
ARC	4-5	19.2 ± 2.9	0.41 ± 0.09	7.8 ± 0.8
	9-10	15.7 ± 2.1	0.55 ± 0.08	8.7 ± 0.7
	21-22	8.4 ± 1.6*†	0.22 ± 0.10†	1.9 ± 0.2*†
NA	4-5	9.0 ± 0.9	0.08 ± 0.14	0.7 ± 0.3
	9-10	12.1 ± 2.4	0.67 ± 0.34*	8.1 ± 1.3*
	21-22	5.2 ± 1.0	ND	ND
ME	4-5	11.6 ± 0.9	0.18 ± 0.08	2.1 ± 0.2
	9-10	14.8 ± 2.2	0.27 ± 0.08	4.1 ± 0.4*
	21-22	10.7 ± 1.7	0.98 ± 0.10*†	10.5 ± 0.4*†
NSO	4-5	16.3 ± 2.4	ND	ND
	9-10	17.3 ± 2.2	ND	ND
	21-22	15.5 ± 3.3	ND	ND
NVM	4-5	11.7 ± 1.4	ND	ND
	9-10	16.1 ± 1.5	ND	ND
	21-22	11.9 ± 1.9	ND	ND

*$p < 0.05$ vs 4-5 mo.; † $p < 0.05$ vs 9-10 mo.; ND = Rate Constants and Turnover Rates could not be accurately determined (see text)

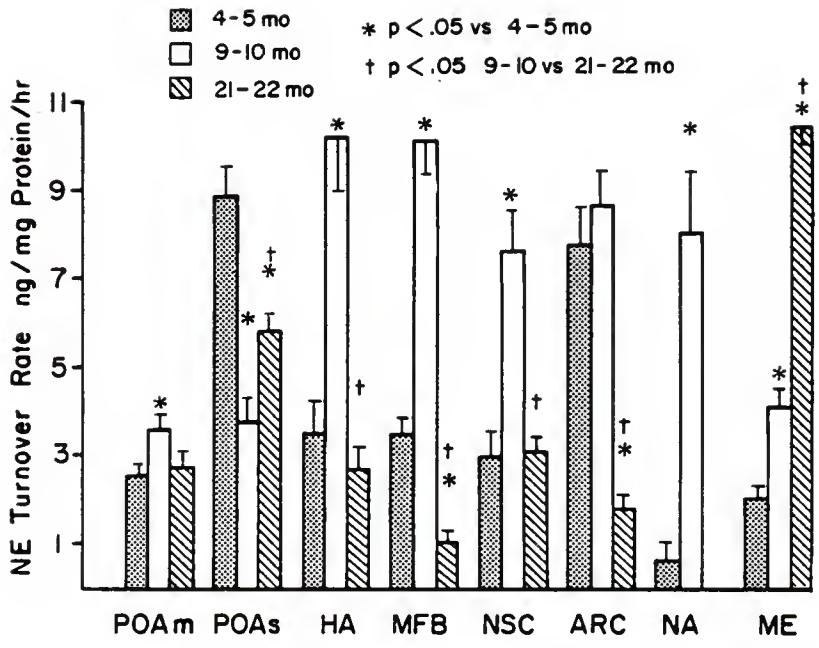

Figure 2. Age-Related Changes in Norephinephrine (NE) Turnover Rates in Microdissected Brain Regions of Ovariectomized Fischer 344 Rats. Each column represents mean turnover rate as determined by a non-steady state method employing 24-30 rats. Only those regions for which turnover rates could be accurately determined are included. Abbreviations used are the same as in Table II.

NE loss since initial concentrations of NE changed little between 4-5 and 9-10 months of age (Table VI). In contrast, the decline in turnover rates seen in the POAs, MFB, NHA, and ARC was due either to a decline in initial NE concentrations (POAs and NHA) or to a decline in both components of turnover rate (MFB and ARC).

Discussion. The results of this study clearly demonstrate that with increasing postmaturational age, regional alterations in concentrations, activities and turnover rates of DA and NE are observed in the ovariectomized F344 rat. Other studies, which have examined larger brain regions, have demonstrated differential responses of DA and NE neurons to advancing age (see 105, 184). The present study indicates that both DA and NE neurons show disparate responses to age within microdissected regions of the ventral diencephalon. Thus the heterogeneity of CA neuronal aging previously observed in large brain region is apparently existent even within these regions. These results further suggest that ubiquitous loss of CA neurons or a generalized decline in CA activity does not occur in the F344 rat.

The responses of dopaminergic neurons to advancing age are of particular interest since most studies have reported an age-related decline in DA concentration and/or DA turnover rates in the hypothalamus.[215-220] In male rats,[215] as well as female Long-Evans rats,[216] the age-related decline in medial basal hypothalamic (MBH) DA concentrations is associated with a decrease in MBH DA turnover[215,216] as well as a decline in DA levels in pituitary stalk blood.[222,223] The present study demonstrates that in the F344 rat, a decline in DA concentrations in the ME and NIL of the pituitary is associated with an increase in DA neuronal activity in these two regions. Thus an age-related decline in DA levels is not obligatively associated with a decline in the turnover rates of DA in these neurons.

The response of noradrenergic neurons to advancing age appears to be considerably more uniform than the alterations observed in dopaminergic neurons. Of the six regions which showed a substantial decline in NE concentration in the 21-22 month old group, five of these areas showed enhanced NE turnover in the 9-10 month old group. These data suggest that the age-related decline in NE concentration may be caused by a preceding state of hyperactivity of these noradrenergic neurons. In the ME, NE turnover was accelerated in both middle-aged and old rats and NE concentrations were stable with age. In the POAs, while a decline in NE concentration in the old group of rats occurred without an acceleration in NE activity in middle-aged animals, it is interesting to note that NE turnover was extremely high in the POAs of young animals. Collectively, these data suggest that a period of chronic hyperactivity of NE neurons may lead to subsequent depletion of NE stores with advancing age.

The PP state can be induced in young rats by electrolytic destruction of the POAm[259] or the OVLT.[260] While we were unable to demonstrate any alteration in CA concentration or turnover in the POAm in old rats, a substantial decline in both DA and NE metabolism was observed in the POAs, and a decline in DA turnover rate was seen in the OVLT. Since large electrolytic lesions of the POAm are likely to include the POAs, it is possible that the CA deficiencies observed in the OVLT and/or POAs may contribute to PP state. This idea is consistent with the observation that local implantation of the CA precursor L-dopa, into the preoptic area,[131] or systemic administration of the dopamine agonist, lergotrile mesylate[179,259] reinitiates normal estrous cycles in old PP rats. However, it is not certain whether the ability of these drugs to reinitiate estrous cycles in PP rats is due to an action in the central nervous system or to their suppression of PRL secretion.

Response of LHRH Neurons to Ovariectomy in Microdissected Brain Regions of Aging Fischer 344 Rats

Objectives. The results of previous reports which examined the effects of age on LHRH concentrations in intact rats are equivocal in that increased,[232] decreased,[220,233,234] or stable[235] values were reported in old versus young animals. The present study was undertaken to characterize LHRH concentrations within regions of the preopticotuberal pathway and to examine the ability of LHRH neurons within these regions to respond to the stimulatory effects of ovariectomy in young, middle-aged and old F344 rats.

Materials and Methods. Barrier-reared F344 rats were purchased at 2-3, 8-9 and 20-21 months of age. Daily vaginal lavages were obtained for 30 days to assess the reproductive state of each animal. Normally cycling young (4-5 months old) and middle-aged (10-11 months old) and PP old (22-23 months old) rats were selected for study on the day of diestrus or two weeks following ovariectomy. Animals were killed by decapitation, trunk blood was collected and necropsies were performed as described above.

Eight brain regions were microdissected and tissues were homogenized in 150 µl of 0.1 N HCl for subsequent LHRH assay. The intraassay coefficient of variation was 6.9% as determined from 12 replicate standard tubes which displaced about 50% of labelled hormone. Minimum LHRH assay sensitivity was established as 2 pg/tube at which 10% of total labelled binding was displaced. Luteinizing hormone releasing hormone was detectable in all samples with the exception of 8% of the OVLT samples. These samples were assigned LHRH levels at minimum assay sensitivity for statistical analysis. Concentrations of LHRH are expressed in terms of pellet protein measured by the dye binding method of Bradford.[246]

TABLE VII. Body and Organ Weights and Health Status of Fischer 344 Rats Employed in Luteinizing Hormone Releasing Hormone Studies

		Age (months) 4-5	10-11	22-23
Number of Rats		20	20	20
Body Weight (g, B.W.)		185.3 ± 2.2	207.5 ± 3.2	277.5 ± 5.3*†
Anterior Pituitary	Weight (mg)	10.9 ± 0.5	12.1 ± 0.6	12.1 ± 0.5
	mg/100 g, B.W.	5.9 ± 0.3	5.9 ± 0.3	4.4 ± 0.2*
Adrenal	Weight (mg)	48.5 ± 1.3	52.6 ± 1.6	56.1 ± 1.6*
	mg/100 g, B.W.	26.3 ± 0.7	25.4 ± 0.8	20.3 ± 0.6*
Abnormalities		Number per group		
Pituitary		0	3	7
Lung		0	2	1
Liver		0	0	3

*$p < 0.05$ vs 4-5 month; † $p < 0.05$ vs 10-11 month

Data were analyzed for age effects with ANOVA followed by Student-Neuman-Kuels tests and for response to ovariectomy with t-tests. Probability values of less than 0.05 were considered statistically significant.

Results. Although the F344 rat has been shown to have a lower incidence of pathologies in advanced age and to maintain general robustness compared to other varieties of inbred rats,[261] inclusion of the occasional abnormal or lesioned animal often confounds the results of studies of senescence.[242] For the present experiments, therefore, only animals with robust appearance which maintained body weight throughout the course of the experiment were chosen. As shown in Table VII, body and adrenal weights increased significantly with age, while anterior pituitary weights did not change. However, when organ weights were expressed as proportions of body weight, a significant decrease was noted for both the adrenal and anterior pituitary glands. While the incidence of visible anterior pituitary and liver abnormalities increased in 22-23 month old rats, these alterations were point-like lesions which did not affect organ weight and no differences in serum hormone or brain LHRH concentrations were observed between lesioned and nonlesioned rats.

Luteinizing hormone releasing hormone concentrations were detectable in all eight of the microdissected areas and the expected regional distribution of LHRH was observed (Table VIII).[262] The highest LHRH concentrations were observed in the median eminence (ME) and this area alone showed a significant decrease in LHRH levels in response to ovariectomy. This decrease in LHRH concentration within the ME of ovariectomized rats was consistently about 20% in all three age groups. In all eight regions examined, no age-related differences in LHRH concentrations were detected.

TABLE VIII. Effects of Age and Ovariectomy on Luteinizing Hormone Releasing Hormone (LHRH) Concentrations Within Microdissected Brain Regions of Fischer 344 Rats

Area	Age	LHRH (pg/μg Protein)	
		Diestrous	Ovariectomized
OVLT	4-5 months	0.31 ± 0.09[a]	0.50 ± 0.20
	10-11 months	0.50 ± 0.05	0.47 ± 0.12
	22-23 months	0.34 ± 0.09	0.79 ± 0.19
POAm	4-5 months	0.21 ± 0.03	0.30 ± 0.08
	10-11 months	0.28 ± 0.02	0.20 ± 0.03
	22-23 months	0.26 ± 0.08	0.20 ± 0.02
POAs	4-5 months	1.97 ± 0.44	0.71 ± 0.39
	10-11 months	1.64 ± 0.26	1.55 ± 0.28
	22-23 months	1.28 ± 0.17	1.44 ± 0.21
NHA	4-5 months	0.08 ± 0.01	0.09 ± 0.01
	10-11 months	0.08 ± 0.01	0.08 ± 0.01
	22-23 months	0.13 ± 0.04	0.08 ± 0.01
NSC	4-5 months	1.05 ± 0.15	0.81 ± 0.10
	10-11 months	1.00 ± 0.08	0.92 ± 0.10
	22-23 months	0.81 ± 0.99	0.94 ± 0.13
ARC	4-5 months	2.73 ± 0.77	2.28 ± 0.78
	10-11 months	1.77 ± 0.29	1.75 ± 0.72
	22-23 months	1.96 ± 0.48	2.13 ± 0.69
NA	4-5 months	1.53 ± 0.30	0.95 ± 0.18
	10-11 months	2.08 ± 0.39	0.99 ± 0.35
	22-23 months	1.41 ± 0.33	1.53 ± 0.43
ME	4-5 months	112.8 ± 4.7	87.8 ± 8.3*
	10-11 months	135.0 ± 8.8	102.9 ± 5.5*
	22-23 months	113.4 ± 7.0	92.0 ± 4.8*

[a]Mean ± SEM; *Significantly less than diestrous $p < 0.05$

Discussion. The major finding in this study is the observation that the PP state in aged F344 rats occurs concomitantly with the maintenance of LHRH concentration along the preopticotuberal pathway and a normal postcastration LHRH decline in the ME. In view of the capacity of these PP rats to ovulate, albeit less frequently than NC younger animals, it is apparent that the two year old F344 rat can respond relatively normally to signals for LH hypersecretion. These data are consistent with the observed normal LH secretion response to a stimulatory regimen of gonadal steroids in the aged PP rat of other strains[162] and the postcastration LHRH depletion response reported in the MBH of middle-aged PP Sprague-Dawley rats.[234]

The results of this study, in conjunction with the previous study which showed that the old ovariectomized F344 maintains NE turnover at levels equal to or greater than those observed in young rats in several regions of the preopticotuberal pathway including the POAm, NHA and ME, indicate that the primary locus of alteration leading to the PP state in old F344 rats is not an impaired responsiveness of LHRH neurons. It is further suggested that a primary defect does not occur in the ability of NE neurons to modulate LHRH neuronal function.

Changes in Catecholamine Activity in Microdissected Brain Regions of Aging Ovariectomized Long-Evans Rats

Objectives. At least two lines of evidence suggest changes in central CA neuronal function may be causally related to altered gonadotropin secretory responses in old rats. First, several stimuli which augment central CA systems are able to restore near normal ovarian cyclic activity in old CE rats (see 105). Second, studies which compared hypothalamic CA concentrations[113,194] and metabolism[145,216] in old male and CE rats reported decreases with age. These decreases in CA neuronal function have been associated with dampened LH response to centrally mediated stimuli[156-159,161-163] and persistent hyperprolactinemia[113,223] in CE rats.

The present study was designed to characterize age-associated alterations in CA neuronal activity within microdissected brain regions in ovariectomized Long-Evans rats. Regions selected for evaluation were primarily those along the preopticotuberal pathway and secondly, regions which contain extensive DA nerve terminal fields from various DA neuron systems. Emphasis was placed upon comparison of young and middle-aged animals prior to establishment of the CE state with old CE rats.

Materials and Methods. Daily vaginal lavages were examined for 30 days in three different cohort groups of Long-Evans rats. Young NC (3-4 months old), middle-aged NC (10 months old) and old (20-22 months old) rats which had been in the CE state for six to ten months were ovariectomized. Two weeks later, each cohort age group was divided into three subgroups. Two subgroups of each cohort were treated with αMPT and killed by decapitation 30 and 60 min after drug treatment. The third subgroups received saline injections and were killed 30 min later. Trunk blood was collected for later evaluation of serum LH and prolactin concentrations. Pituitary tissues were removed and homogenized as previously outlined and animals were necropsied for visible abnormalities. Ten brain areas were microdissected and homogenized as described above. Neural tissue homogenates were then assayed for DA and NE concentrations. Minimum sensitivities of the DA assays were approximately 20 pg for DA and 50 pg for NE.

Results. In an attempt to separate the effects of age from effects which might be attributed to pathology, only animals with robust appearance and maintained body weight were selected for study. Additionally, animals were necropsied for gross visible lesions and weights of several organs were recorded. Of the 32 old rats selected for study, five were eliminated from data analysis on the basis of putuitary tumors noted at necropsy.

TABLE IX. Body and Organ Weights and Health Status of Long-Evans Rats Employed in Catecholamine Studies

		Age (months) 3-4	10	20-22
Number of Rats		31	31	27
Body Weight (g, B.W.)		285.3 ± 3.8[a]	338.9 ± 5.8*	411.3 ± 9.2*†
Anterior Pituitary	Weight (mg)	9.2 ± 0.3	10.5 ± 0.4*	13.1 ± 0.6*
	mg/100 g B.W.	3.2 ± 0.1	3.1 ± 0.1	3.2 ± 0.1
Adrenal	Weight (mg)	61.5 ± 2.6	58.0 ± 2.6	66.5 ± 3.0†
	mg/100 g B.W.	21.5 ± 0.9	17.2 ± 0.8*	16.2 ± 0.7*
Kidney	Weight (g)	2.11 ± 0.04	2.46 ± 0.07*	3.13 ± 0.08*†
	mg/100 g B.W.	742.8 ± 15.9	728.2 ± 18.6	768.3 ± 20.2
Heart	Weight (mg)	726.7 ± 13.6	790.1 ± 19.2	966.8 ± 20.6*†
	mg/100 g B.W.	255.0 ± 4.2	233.5 ± 4.7*	236.3 ± 4.5*
Abnormalities			Number per group	
Pituitary		0	0	7
Lung		12	15	6
Kidney		0	1	2
Liver		0	3	6

[a]mean ± SEM; *p < 0.05 versus 3-4 months; † 0.05 versus 10 months

Results recorded at necropsy are summarized on Table IX for animals used in this study. Both body and organ weights tended to increase with age; however, values were stable or decreased with age when expressed as proportions of body weight. Decreased ratios of adrenal and heart weights may be attributed to increased proportions of body fat with age; however, these ratios did not change between 10 and 20-22 month old rats. The incidence of pituitary, liver and kidney abnormalities increased with age. These abnormalities consisted primarily of small point-like lesions and no consistent trends in hormone or CA levels were apparent between lesioned and nonlesioned animals included in data analysis.

Steady state DA concentrations (measured in saline treated rats) tended to decrease with age in seven of nine brain regions examined for this amine (Table X). Decreased DA concentrations in regions from old versus young rats were most dramatic in the NAc (34%), NHA (54%), NIL (51%), and ME (74%). Concentrations of DA decreased significantly between 3-4 and 10 months of age only in the NHA (31%) and ME (40%).

Steady state NE concentrations similarly tended to decrease from 23 to 59% in old versus young rats in the regions examined (Table XI). Decreases were significant in the POAs (54%), MFB (44%), NSC (49%), and ME (59%). Concentrations of NE in 10 month old rats were significantly diminished compared to young rats only in the NSC (46%) and ME (40%).

Turnover rates of DA were decreased substantially in five regions from old versus young animals (Table X). These regions were the POAm (45%), POAs (59%), NHA (81%), ME (63%) and NIL (48%). Interestingly, DA turnover rates increased in old compared to middle-aged and/or young rats in the NAc, striatum and NA. The increases in DA turnover in these three regions were attributed to augmented neuronal activity as reflected by DA rate

TABLE X. Age-Related Change in Dopamine Activity Within Microdissected Brain Regions of Ovariectomized Long-Evans Rats

Area	Age (months)	[DA] initial (ng/mg protein)	Rate Constant (K)	Turnover Rate (ng/mg protein/hr)
NAc	3-4	233.9 ± 13.4	0.53 ± 0.09	123.5 ± 5.7
	10	180.9 ± 18.8	0.48 ± 0.09	87.5 ± 6.0*
	20-22	154.7 ± 11.1*	1.13 ± 0.08*†	174.8 ± 7.8*†
S	3-4	227.0 ± 15.4	0.30 ± 0.08	68.5 ± 4.3
	10	254.0 ± 12.0	0.11 ± 0.07	29.2 ± 3.3*
	20-22	226.0 ± 15.7	0.36 ± 0.11	81.7 ± 5.8†
POAm	3-4	14.4 ± 2.6	0.48 ± 0.13	6.9 ± 0.8
	10	14.3 ± 1.8	0.59 ± 0.12	8.5 ± 0.7
	20-22	9.3 ± 0.6	0.41 ± 0.12	3.8 ± 0.3*†
POAs	3-4	24.3 ± 3.2	1.03 ± 0.17	24.9 ± 2.1
	10	24.6 ± 5.1	0.92 ± 0.14	22.8 ± 2.9
	20-22	24.7 ± 2.8	0.41 ± 0.13*	10.1 ± 0.9*†
NHA	3-4	10.8 ± 1.2	0.53 ± 0.16	5.8 ± 0.5
	10	7.4 ± 0.8*	0.40 ± 0.10	2.9 ± 0.2*
	20-22	5.0 ± 0.9*	0.21 ± 0.15	1.1 ± 0.2*†
NVM	3-4	2.4 ± 0.6	0.33 ± 0.19	0.8 ± 0.1
	10	1.7 ± 0.2	0.38 ± 0.16	0.7 ± 0.3
	20-22	1.8 ± 0.2	0.50 ± 0.14	0.9 ± 0.1
NA	3-4	43.7 ± 5.4	1.00 ± 0.14	43.9 ± 1.2
	10	33.6 ± 5.7	0.77 ± 0.14	26.0 ± 2.7*
	20-22	29.9 ± 4.1	1.32 ± 0.15†	39.6 ± 3.1†
ME	3-4	210.9 ± 22.4	0.83 ± 0.11	175.9 ± 11.8
	10	125.4 ± 13.8*	0.81 ± 0.10	102.1 ± 7.0*
	20-22	54.4 ± 7.6*†	1.18 ± 0.15	64.4 ± 5.2*†
NIL	3-4	12.5 ± 1.7	0.80 ± 0.12	10.0 ± 0.9
	10	10.0 ± 0.9	0.33 ± 0.22	3.3 ± 0.5*
	20-22	6.1 ± 0.6*	0.91 ± 0.16†	5.2 ± 0.4*

*$p < 0.05$ versus 3-4 month old group; † $p < 0.05$ versus 10 month old group

constants (K). Although rate constants were not significantly different in any region between young and middle-aged rats, they increased significantly in the NAc (135%), striatum (139%), NIL (175%), and NA (71%) and were moderately elevated in the NVM (36%), and ME (45%) between middle- and old-age. Only the POAs region had a significant decrease in DA rate constant in old versus young rats.

As shown in Table XI, turnover rates of NE in microdissected tissues from 20-22 month old rats were significantly decreased compared to 3-4 month old rats in the POAs (98%), NSC (21%), NVM (51%) and ME (38%), while turnover rates were augmented in the POAm (44%) and NHA (85%). Interestingly, for four regions in which decreased NE turnover was observed in old animals, a significant decline in NE turnover was seen in middle-aged rats. Rate constants of NE remained stable although they tended to increase in old compared to younger rats in all regions except the POAs. Thus, as in the case for DA, decreases in NE turnover rates observed in several brain regions of old animals were primarily the result of decreased NE concentrations rather than neuron activity. In contrast, regions which showed a decreased NE turnover in middle-aged animals, both a decline in NE concentration and K,appeared to contribute to the turnover decreases.

We noted that regions with relatively high NE rate constants in young rats were those regions which showed the greatest NE concentration depletion in old animals. When this relationship was analyzed, a strong positive correlation was found between neuronal activity (K) measured in regions from young animals and the magnitude of the NE depletion measured in corresponding regions from old animals ($r = 0.72$). Similarly, the relationship between NE activity in young animals and percent NE depletion calculated between 4-5 and 10 month old rats was positively correlated ($r = 0.84$).

TABLE XI. Age-Related Change in Norepinephrine Activity Within Microdissected Brain Regions of Ovariectomized Long-Evans Rats

Area	Age (months)	[NE] initial (ng/mg protein)	Rate Constant (K)	Turnover Rate (ng/mg protein/hr)
POAm	3-4	28.1 ± 2.9	0.27 ± 0.08	7.7 ± 0.6
	10	24.0 ± 3.3	0.39 ± 0.11	9.4 ± 0.9
	20-22	21.7 ± 1.9	0.51 ± 0.11	11.1 ± 0.8*
POAs	3-4	50.0 ± 7.9	0.63 ± 0.13	31.5 ± 3.1
	10	33.8 ± 6.4	0.38 ± 0.15	12.8 ± 1.7*
	20-22	22.9 ± 3.3*	0.03 ± 0.17*	0.6 ± 0.8*†
NHA	3-4	10.2 ± 1.1	0.13 ± 0.12	1.3 ± 0.2
	10	11.2 ± 1.0	0.53 ± 0.10	5.9 ± 0.4*
	20-22	7.7 ± 1.0	0.31 ± 0.14	2.4 ± 0.3*†
MFB	3-4	18.8 ± 1.8	0.49 ± 0.11	9.2 ± 0.7
	10	14.4 ± 1.3	0.86 ± 0.15	12.4 ± 0.8
	20-22	10.6 ± 4.4	0.57 ± 0.18	6.1 ± 1.5†
NSC	3-4	18.9 ± 1.6	0.59 ± 0.13	11.1 ± 0.7
	10	10.2 ± 1.2*	0.00 ± 0.14*	0
	20-22	9.6 ± 1.1*	0.91 ± 0.15†	8.8 ± 0.6*
NVM	3-4	11.0 ± 2.1	0.41 ± 0.19	4.5 ± 0.6
	10	7.4 ± 0.8	0.16 ± 0.15	1.2 ± 0.2*
	20-22	6.9 ± 0.8	0.32 ± 0.14	2.2 ± 0.2*
NA	3-4	37.0 ± 2.5	0.48 ± 0.13	17.7 ± 1.1
	10	29.2 ± 7.9	0.58 ± 0.16	16.9 ± 2.8
	20-22	29.0 ± 4.2	0.42 ± 0.14	12.3 ± 1.3
ME	3-4	29.8 ± 4.4	0.50 ± 0.14	15.0 ± 1.5
	10	18.0 ± 2.1*	0.26 ± 0.12	4.8 ± 0.5*
	20-22	12.2 ± 1.7*	0.76 ± 0.23	9.3 ± 0.9*†

*$p < 0.05$ vs 3-4 month old group; † $p < 0.05$ vs 10 month old group

In contrast, no correlation was detected when a similar comparison of DA concentration and neuronal activities was evaluated ($r = 0.05$).

Discussion. The results of this study clearly demonstrate that CA concentrations and neuron activities within microdissected regions along the preopticotuberal pathway in ovariectomized Long-Evans rats are differentially changed with age. Collectively, this study and our results discussed above from male Wistar and ovariectomized F344 rats extend and confirm earlier reports which examined the effects of age on CA function in large brain regions from rodents and humans.[184-190] Thus, age-associated changes in central CA function are not uniform in their magnitude or direction within brain areas. Age-associated alterations are relatively focalized and appear to be maximal within specific nerve terminal fields. The importance of this study and the previous study in ovariectomized F344 rats lies in the observation that CA neuron activities are not generally decreased with age but, rather, are augmented in some brain regions from old compared to younger animals.

A rather progressive age-related decline in NE concentrations was observed in the eight brain regions examined in the present study. Since NE neuronal activity (K) decreased significantly in only the NSC and POAs of middle-aged and old animals, a progressive decline in the activity of NE neurons cannot account for the loss of stores of this CA. Rather, the age-related decline of NE concentrations appears to be the primary contributor to the substantial decline in NE turnover observed in the NSC, NVM, and the ME. Interestingly, an increase in NE turnover was observed in the POAm and the NHA in old ovariectomized rats. This increase resulted from enhanced neuronal activity (K). Thus, the age-related loss of NE stores rather than a decline in the activity of neurons appears to be the primary contributor

to the decline in NE turnover observed in several hypothalamic regions. A partial explanation for the divergent effects of age on NE turnover may be the activity of the NE neurons. An interesting positive correlation between NE neuronal activity in young animals and the magnitude of the age-related loss of NE stores was observed. Thus, initial high neuronal activity was associated with a loss of NE and a decline in NE turnover. In contrast, initial low NE activity in regions from young animals was associated with the maintenance of NE concentration and an increase in NE turnover in corresponding areas from old rats. In view of the evidence that stimuli which acutely accelerate NE turnover cause moderate depletion of NE stores,[263] it is not unreasonable to suggest that persistent hyperactivity of NE neurons may contribute to the age-related loss of NE stores. In this regard, in the F344 rat, the loss of NE stores between middle-aged and old rats was associated by an increase in NE neuron activity in younger animals.

The observation that in five of six regions in which DA concentrations were reduced in old rats, DA turnover rates were also reduced indicates that, as for NE neurons, reduced DA stores are a primary contributor to the age-related decline in DA turnover. Neuronal activity of DA was reduced with age only in the POAs and was significantly increased with age in the NAc, striatumand the NA. The enhanced neuronal activity in these latter three DA regions indicates that some DA systems retain into late life the capacity to respond to DA loss with enhanced activity, a capacity clearly established for CA neurons in young animals.[93] However, for most brain regions, DA neuronal hyperactivity is not sufficient to compensate for the dramatic loss of DA stores as this is reflected in a decline of DA turnover.

The roles of CA neurons in the regulation of AP hormone secretion have been studied extensively. The significant decreases in NE turnover detected in five of eight regions examined along the preopticotuberal pathway from old versus young ovariectomized Long-Evans rats support the concept that NE neuronal dysfunction with age is related to impaired LH hypersecretion responses in old CE rats previously proposed.[113] However, examination of NE turnover from ovariectomized previously NC middle-aged rats indicates that a progressive age-related decrease in NE turnover does not occur in most hypothalamic regions. Whether decreases in NE turnover detected in some regions from middle-aged ovariectomized rats may be related to the diminished preovulatory surge of LH reported in these animals[152-155] is unclear. Interestingly, a reduced NE response to stimulatory regimens of gonadal steroids has been reported in middle-aged animals prior to the onset of CE.[264]

Decreased DA concentrations and DA turnover in MBH tissues of old male and CE rats appear to be associated with hyperprolactinemia.[113,145,216] Although DA turnover rates were progressively decreased with age in ovariectomized Long-Evans rats in the ME, serum prolactin levels were not elevated in old ovariectomized rats.[159,161,175] Thus, TIDA neuron activity, although reduced in old ovariectomized Long-Evans rats, appears sufficient to prevent prolactin increases. The results of the present study support the concept that hyperprolactinemia in the CE rat is the result of chronically increased estrogen levels.[161] In contrast to the preceding similar study in F344 rats, TIDA neurons terminating in the ME of old Long-Evans rats do not maintain DA turnover rates in the presence of decreased DA concentrations. Since hyperprolactinemia has been shown to reduce DA concentrations in MBH regions of young rats,[92] elevated prolactin levels, previously maintained in these

intact CE rats, may have significantly contributed to the decreased DA concentrations.

Response of LHRH Neurons to Ovariectomy within Microdissected Brain Regions of Aging Long-Evans Rats

Objectives. Results of several studies have shown that the post-castration rise in LH and the LH response to stimulatory regimens of gonadal steroids, both CNS mediated events, are attenuated in old CE rats.[144,149,156,157-159,161-163] Because similar LH secretion patterns are observed in young and old CE rats after appropriate treatment with LHRH, a CNS defect appears primarily responsible for dampened LH responses in these CE rats.[127] Results of previous studies which examined LHRH concentrations in aging rats are equivocal in that increased,[232] decreased,[220,233,239] or unchanged[235] levels were reported in CE old versus young rats. Although the preceding experiments suggested diminished NE turnover in several areas of old ovariectomized rats may contribute to impaired LH secretion, the extent of decreased NE turnover was similar in some regions of middle-aged rats which exhibited normal estrous cycles at the time of ovariectomy. Age-related alterations in LHRH neuronal function might clarify the locus of impairment leading to the CE state. The purpose of the present study was first to characterize the effects of age on LHRH concentrations within regions along the preopticotuberal pathway and second, to examine the ability of these neurons to respond to the stimulatory effects of ovariectomy. Because of the lack of methods to more directly measure LHRH neuron activity, LHRH concentration depletion after ovariectomy was used as an index of LHRH neuron function.

Materials and Methods. After 30 days, during which reproductive cycles were monitored, NC young (3-4 month old), IC middle-aged (7-8 month old) and CE old (20-24 month old) Long-Evans rats were selected for study.

Middle-aged rats were classified as IC if they consistently had 3-5 consecutive days of cornified vaginal epithelium with two or three days of leukocytic smears during the evaluation period. A subgroup of rats from each cohort group was ovariectomized. Rats were killed by decapitation on the morning of estrus or two weeks following ovariectomy. Another subgroup of 3-4 month old rats was killed on the morning of diestrus. Brains were removed and frozen, trunk blood was collected and animals were necropsied as described above. Seven areas along the preopticotuberal pathway were microdissected, homogenized in 100 µl of 0.1 N hydrochloric acid and assayed for LHRH content. The intraassay coefficient of variation was 11.5% as determined from 11 replicate standard tubes which displaced about 50% of labelled hormone. Minimum assay sensitivity at which 10% of labelled hormone was displaced was 3.7 pg. Supernatants from NA and ME tissues were assayed in duplicate. Data were analyzed for age effects with analysis of variance followed by Student Neuman-Kuels tests and for response to ovariectomy with t-tests. Probability values of less than 0.05 were considered statistically significant.

Results. Table XII summarizes the health status of the animals used in this study. As previously found in Long-Evans rats in our colony, both body and organ weights tended to increase with age. Organ weights remained stable or decreased in old animals when expressed as proportions of body weight. Although the incidence of visible abnormalities increased with age, no consistent trends in hormone levels were apparent between lesioned and nonlesioned rats.

The expected regional distribution of LHRH was observed among the seven microdissected regions,[262] as shown in Table XIII. Although LHRH levels tended to decrease from 13 to 27% in ME tissues from ovariectomized

TABLE XII. Body and Organ Weights and Health Status of Long-Evans Rats Employed in Luteinizing Hormone Releasing Hormone Study

		Age (months) 3-4	7-8	20-24
Number of Rats		30	20	21
Body Weight (g, B.W.)		272.5 3.8	335.0 7.9	430.8 ± 12.8
Anterior Pituitary	Weight (mg)	10.4 ± 0.3	11.8 ± 0.7	14.3 ± 0.9
	mg/100 g B.W.	3.8 ± 0.1	3.7 ± 0.2	3.4 ± 0.3
Adrenal	Weight (mg)	56.8 ± 1.2	54.0 ± 1.7	67.6 ± 3.2
	mg/100 g B.W.	20.9 ± 0.5	16.2 ± 0.6	15.8 ± 0.6
Kidney	Weight (g)	1.89 ± 0.03	2.13 ± 0.07	3.04 ± 0.08
	mg/100 g B.W.	696.4 ± 13.2	643.4 ± 18.6	718.2 ± 27.9
Heart	Weight (mg)	758.8 ± 11.1	855.5 ± 16.8	1145.9 ± 23.2
	mg/100 g B.W.	279.1 ± 4.1	255.8 ± 5.6	268.3 ± 5.5
Abnormalities			Number per group	
Pituitary		0	0	8
Lung		10	5	7
Kidney		0	0	6
Liver		0	0	4

TABLE XIII. Effect of Age and Ovariectomy on Luteinizing Hormone Releasing Hormone Concentrations Within Microdissected Brain Regions of Long-Evans Rats

Area	Age (months)	LHRH (pg/μg protein) Estrous	Diestrous	Ovariectomized
OVLT	3-4	0.68 ± 0.11	0.82 ± 0.17	0.88 ± 0.20
	7-8	1.74 ± 0.74		1.42 ± 0.76
	20-24	0.62 ± 0.19		1.48 ± 0.72
POAs	3-4	0.75 ± 0.30	0.96 ± 0.24	1.36 ± 0.45
	7-8	0.93 ± 0.24		1.40 ± 0.37
	20-24	1.22 ± 0.25		1.33 ± 0.35
POAm	3-4	0.03 ± 0.00	0.03 ± 0.00	0.07 ± 0.01
	7-8	0.03 ± 0.00		0.05 ± 0.01
	20-24	0.05 ± 0.01		0.06 ± 0.01
NSC	3-4	0.32 ± 0.05	0.40 ± 0.06	0.62 ± 0.06
	7-8	0.26 ± 0.03		0.34 ± 0.03
	20-24	0.38 ± 0.08		0.38 ± 0.06
ARC	3-4	3.37 ± 1.38	2.31 ± 0.53	1.08 ± 0.44
	7-8	1.36 ± 0.45		2.14 ± 0.78
	20-24	0.86 ± 0.21		2.47 ± 0.54
NA	3-4	1.80 ± 0.42	1.62 ± 0.48	1.12 ± 0.20
	7-8	1.10 ± 0.29		0.85 ± 0.17
	20-24	1.97 ± 0.67		2.29 ± 0.77
ME	3-4	47.3 ± 4.7	47.0 ± 4.8	41.1 ± 3.6
	7-8	64.3 ± 5.9*		46.6 ± 7.1
	20-24	57.3 ± 4.8		43.0 ± 4.4

*significantly different from 3-4 month old groups $p < 0.05$

compared to intact rats of each age group, these differences were not significant at the 95% confidence level. Only one significant effect of age was detected in any area of intact or ovariectomized rats examined. Concentrations of LHRH in the ME were increased in middle-aged compared to young estrous rats. No significant age-related changes in LHRH concentrations were observed among brain areas in either intact or ovariectomized rats.

Discussion. The results of the present study show that LHRH concentrations along the preopticotuberal pathway in both intact and ovariectomized Long-Evans rats are not significantly changed during advanced age. Thus, the severely impaired LH response of CE rats to ovariectomy[156-158] and to stimulatory regimens of gonadal steroids[161-163] cannot be explained by inadequate amounts of hypothalamic LHRH. Either impaired LHRH neurosecretion and/or altered AP response to LHRH might contribute to dampened LH secretion response in these animals. This latter possibility was supported by studies which observed decreased LH response after a single injection[127] or during continuous infusion[166] of LHRH in CE rats. However, the similar LH secretion profiles observed in both old CE and young rats after repeated LHRH administration indicates AP response to LHRH is not severely altered during advanced age.[127]

Although the LHRH depletion response to ovariectomy has been used as an index of neurohormone secretion[262] and the present study found no effects of age on LHRH response to castration, these results must be interpreted with caution. The primary limitation of this method is that it does not reflect the mode of LHRH secretion. Since several studies have shown that the pattern of LHRH stimulation affects the LH secretion response,[28,29] diminished LH release observed in old CE rats may result from altered modes

of LHRH secretion. Specifically, either the amplitude or frequency of LHRH release may be altered in the old animal. Additionally, this method cannot distinguish between altered synthesis and release rates of LHRH. Depletion response measured 2-3 weeks after castration suggests that synthesis of the peptide is insufficient to compensate for increased release rates in the young animal.[262] Although the magnitude of LHRH depletion response was similar in young and old animals in the present study, changes in the regulatory mechanisms which maintain LHRH synthesis and release rates could be altered with age. While results of this study clearly show hypothalamic LHRH concentrations are not altered in old CE rats, further studies are required to clarify whether LHRH release mechanisms are impaired in these animals.

Age-Related Alterations in Luteinizing Hormone and Prolactin Response to Ovariectomy and α-Methylparatyrosine

Changes with Age in Serum Luteinizing Hormone and Prolactin Levels in Fischer 344 Rats

Objectives. Basal levels of LH have generally been reported to remain stable with age in several species.[114,148,149] In contrast, the response of LH to stimulatory regimens of gonadal steroid in old rats appears to depend upon the stage of reproductive senescence. Steroid-induced hypersecretion of LH is consistently decreased in old CE rats,[156-159] while in old PP rats it is reported to be normal[162] or dampened.[161] The castration-induced hypersecretion of LH is consistently dampened in old rats.[144,156,161-163] Thus, the mechanisms regulating LH hypersecretion appear to be affected to varying extents in the CE and PP old rat.

Increases in serum prolactin levels during advanced age are probably the best characterized age-associated changes in AP hormones (see 113). However, the mechanisms responsible for prolactin elevations also appear to

differ with reproductive status of old rats. Ovariectomy decreases prolactin in CE rats to levels similar to young animals,[159,161,175] but has little effect on prolactin levels in old PP rats.[161,175]

The present studies examined serum LH and prolactin levels and monitored pituitary concentrations of these hormones from F344 rats used in experiments designed primarily to evaluate CA and LHRH neuronal alterations with age. Serum hormone levels were evaluated in an attempt to characterize the magnitude of age-associated effects on AP secretion in the animals employed in these studies. Characterization of these concentrations also provided a basis for comparison with previously reported results.

Materials and Methods. Prolactin and LH concentrations were evaluated by standard RIA methods in sera and AP tissues from F344 rats employed in the previous studies of CA metabolism in ovariectomized rats and LHRH concentration response to ovariectomy.

Results. Serum LH concentrations measured in each of three age groups of ovariectomized F344 rats killed 0, 45 and 90 min after treatment with αMPT are shown in Figure 3. There were no differences in LH concentrations between age groups in saline treated rats (0 min after αMPT). Levels of LH tended to increase in both young and middle aged rats after drug treatment, but no significant differences were detected between saline and αMPT treated rats of these two age groups. In marked contrast, 21-22 month old rats had significantly decreased LH levels after drug administration. These decreases were significant compared to both younger groups of drug treated animals and old saline treated rats.

Serum prolactin levels were elevated about four-fold in old compared to younger saline treated ovariectomized rats as shown in Figure 4. Animals in all age groups responded to αMPT with increased serum prolactin

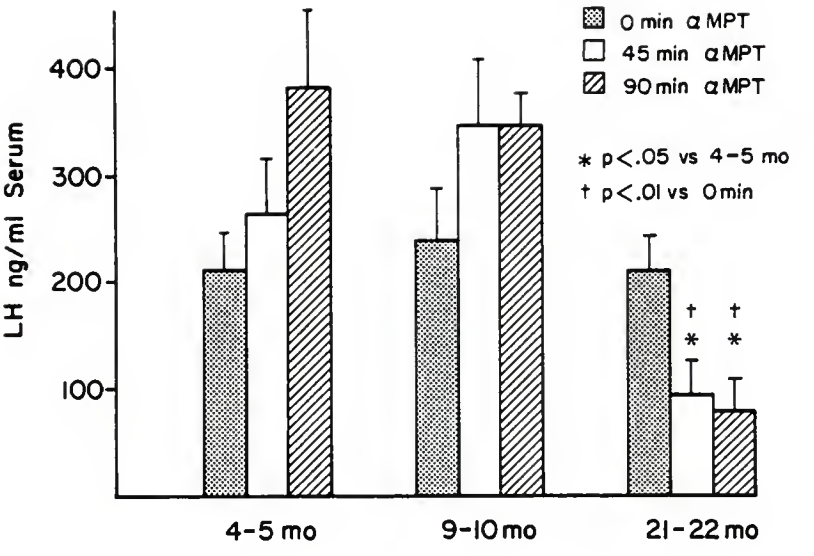

Figure 3. Age-Related Changes in Serum Luteinizing Hormone (LH) Concentrations After α-Methylparatyrosine (αMPT) in Ovariectomized Fischer 344 Rats. Columns represent mean hormone concentrations of 9-10 animals at 0, 45 or 90 min after αMPT (250 mg/Kg, ip) while bars represent SEM.

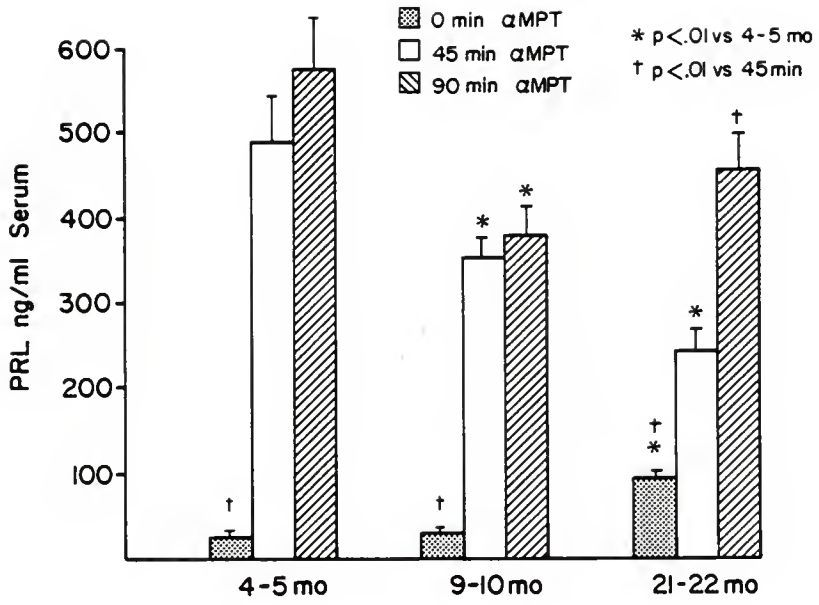

Figure 4. Age-Related Changes in Serum Prolactin (PRL) Concentrations After α-Methylparatyrosine (αMPT) in Ovariectomized Fischer 344 Rats. See Figure 3 for further explanation.

concentrations; however, the hormone response to drug treatment was delayed in 21-22 month old rats.

The effects of ovariectomy in the three cohort groups of F344 rats studied on serum LH and prolactin levels are shown in Tables XIV and XV, respectively. Although basal LH concentrations in intact animals tended to increase in middle-aged compared to young diestrous and old PP rats, these differences were not significant. Values for intact 4-5 and 22-23 month old animals were often below sensitivity limits of the assay. Consistent with the data on Figure 3, no significant differences between age groups were detected in ovariectomized rats. Prolactin levels were significantly increased by more than three-fold in all PP rats compared to younger diestrous animals (Table XV). Serum prolactin levels did not decrease in response to ovariectomy in old rats, but were decreasedin younger ovariectomized rats compared to their diestrous cohorts.

Discussion. The results of these studies clearly show that the F344 rat maintains a normal postcastration LH response to ovariectomy through advanced age. Together with the results of the preceding study which showed these animals maintain normal LHRH concentrations and depletion response to ovariectomy, these data indicate that LHRH neurons are able to function in a relatively normal manner. This conclusion is supported by the unimpaired LH response to stimulatory regimens of gonadal steroids previously reported in old PP rats.[162] Although LHRH depletion response to ovariectomy has been shown in the MBH of PP Sprague-Dawley rats,[234] a diminished LH postcastration response has been described in Long-Evans, Wistar and Sprague-Dawley strains of rats following establishment of the PP state.[161,179,234] These apparent discrepancies in LH secretion response between PP rats may possibly be attributed to the strain of rat or the possible presence of the CE state

TABLE XIV. Effects of Ovariectomy on Serum Luteinizing Hormone Concentrations in Aging Fischer 344 Rats

Age	Serum LH (ng/ml) Diestrous	Ovariectomized	% Increase
4-5 months	21.8 ± 6.7(10)[a]	370.8 ± 63.2(11)	1600
10-11 months	55.5 ± 16.3(10)	340.5 ± 50.0(10)	514
22-23 months	8.1 ± 1.8(10)*[b]	244.3 ± 32.5(10)	2916

[a]Mean ± SEM (no./group); [b]six of 10 animals < detectable at the serum volume used. These six animals were therefore assigned values of 5 ng/ml, the lower limits of sensitivity of the LH assay at the volumes employed (200 µl serum); *$p < 0.05$ versus 10-11 months.

TABLE XV. Effects of Ovariectomy on Serum Prolactin Concentrations in Aging Fischer 344 Rats

Age	Serum PRL (ng/ml) Diestrous	Ovariectomized	% Decrease
4-5 months	34.8 ± 10.2(10)[a]	9.0 ± 2.7(10)†	74
10-11 months	37.1 ± 12.4(10)	7.9 ± 1.0(10)†	79
22-23 months	119.7 ± 21.3(8)*	97.6 ± 18.5(10)*	18

[a]Mean ± SEM (no./group); *significantly greater than 4-5 and 10-11 groups. $p < 0.01$; †significantly less than diestrous. $p < 0.05$

in these other strains of rats. Decreased AP sensitivity to LHRH has also been suggested since old Long-Evans and Sprague-Dawley PP rats have dampened LH secretion after a single injection of LHRH[127] and continuous LHRH infusion[166] compared to young animals. However, the normal LH profiles observed in PP rats after repeated LHRH injections[127] indicate AP response is probably not severely altered in the PP state. Since the incidence of age related pathologies was not mentioned in previous reports, this could also contribute to disparate LH secretion response observed between this and other studies.

Serum LH concentrations in ovariectomized αMPT treated rats revealed some very curious age-associated differences. The maintained LH levels observed in young and middle-aged drug treated rats are consistent with studies which showed pulsatile LH secretion was not altered after αMPT treatment.[69] The marked decrease of LH in drug treated old animals suggests that LHRH neurons may have altered dependence on CA activity in old PP rats. Correlative evidence which supports this is the augmented NE activity in the ME of old versus young F344 rats shown in the preceding study. Whether augmented CA function is required for maintained LHRH function in the old F344 rat should be examined.

The elevated serum prolactin levels measured in both intact and ovariectomized old rats are consistent with previous studies which showed hyperprolactinemia in old PP rats was not altered after removal of ovarian steroids.[161] The enhanced DA turnover rates in the ME of these old animals suggest a decline in AP response to the inhibitory effects of DA may be causally related to hyperprolactinemia in PP rats. Alternatively, age-associated decreases in another prolactin inhibiting factor might also explain augmented serum prolactin levels in old PP rats. If dampened AP

sensitivity to the prolactin inhibiting effects of DA contribute to hyperprolactinemia in PP rats, the increased prolactin levels in αMPT treated rats of all ages indicate that the magnitude of these alterations is probably not great. The ability of old ovariectomized rats to respond to αMPT indicates that prolactin in these animals is maintained at relatively low rates of secretion compared to the AP capacity. Thus, the accelerated TIDA activity in these animals does dampen prolactin secretion. Prolactin response to αMPT was delayed in 21-22 month old animals. This sluggish response might result from augmented TIDA activity or AP sensitivity changes.

Collectively, these hormone, LHRH and CA data indicate that alterations which contribute to the PP state in the old F344 rat do not primarily involve LH secretion response regulation. The chronic elevation of serum prolactin which is associated with increased TIDA activity in these rats suggests that the nature of impaired prolactin regulation may involve decreased AP sensitivity to DA. Whether prolactin elevations contribute to the onset or maintenance of the PP state in these animals should be evaluated.

Changes with Age of Serum Luteinizing Hormone and Prolactin Levels in Long-Evans Rats

Objectives. The present studies were designed to evaluate serum LH and prolactin levels in Long-Evans rats employed in studies which characterized the effects of age on CA activity and LHRH response to ovariectomy. Data from these animals were obtained as a basis of comparison with other investigations on old CE rats.

Materials and Methods. Trunk blood was collected at decapitation from Long-Evans ovariectomized rats treated with αMPT or saline and untreated

intact or ovariectomized animals as described above. Sera were evaluated for LH and prolactin concentrations using standard RIA methods as previously described. Prolactin concentrations are expressed in terms of standard reference NIAMDD PRL-RP-2.

Results. Serum LH concentrations were severely dampened in old versus young ovariectomized rats at 0, 30 and 60 min following αMPT treatment (Table XVI). Further, these decreases appeared to be progressive with increasing age since levels in middle-aged animals were between those observed in young and old rats. Concentrations of LH were slightly augmented or unchanged after αMPT treatment in young rats but decreased in middle-aged and old animals.

Prolactin concentrations were not different between age groups in ovariectomized saline treated rats and increased significantly after αMPT in all cohort groups (Table XVI). The response of prolactin to αMPT was delayed in old animals; however, peak prolactin levels were not different between age groups.

Table XVII shows the effects of ovariectomy on serum LH and prolactin levels in three similar age groups of otherwise untreated rats. Levels of LH were increased in young and middle aged ovariectomized rats compared to their respective estrous cohorts. While old rats had lower LH levels after ovariectomy compared to younger rats and LH was not significantly elevated in old ovariectomized rats compared to intact CE rats, LH in estrous rats showed an age-related increase.

Prolactin levels increased in old CE compared to both younger age groups (Table XVII). While prolactin decreased in young and old ovariectomized rats compared to intact animals, there were no age differences between ovariectomized rats.

TABLE XVI. Effect of α-Methylparatyrosine (αMPT) on Serum Luteinizing Hormone and Prolactin Levels in Aging Ovariectomized Long-Evans Rats

Time after αMPT (min)	Age (months) 3-4	10	20-22
		LH (ng/ml)	
0	200 ± 24(11)[a]	140 ± 21(11)	53 ± 10(9)*†
30	307 ± 41(10)	89 ± 32(9)*	Δ20 ± 4(8)*
60	Δ308 ± 30(10)	112 ± 27(10)*	29 ± 7(10)*
		Prolactin (ng/ml)	
0	18 ± 4(11)	26 ± 8(11)	29 ± 8(9)
30	Δ325 ± 53(10)	Δ245 ± 52(10)	Δ187 ± 13(8)
60	Δ166 ± 24(10)	Δ188 ± 25(10)	Δ332 ± 80(10)*

[a]Mean ± SEM (number per group); *$p < 0.05$ versus corresponding 3-4 month old group; †$p < 0.05$ versus corresponding 10 month old group; Δ$p < 0.05$ versus 0 min after αMPT

TABLE XVII. Effects of Ovariectomy on Serum Luteinizing Hormone and Prolactin Levels in Aging Long-Evans Rats

Age (months)	Estrous	Ovariectomized	Diestrous
		LH (ng/ml serum)	
3-4	43.5 ± 6.4(10)[a]	316.8 ± 38.7(10)†	57.7 ± 10.6(10)
7-8	73.0 ± 11.6(10)	282.3 ± 42.3(10)†	
20-24	100.4 ± 9.7(10)*	133.4 ± 18.4(11)*	
		Prolactin (ng/ml serum)	
3-4	60.3 ± 14.1(10)	28.3 ± 3.6(10)†	104.0 ± 35.5(10)
7-8	28.2 ± 6.7(9)	31.8 ± 7.1(10)	
20-24	128.5 ± 18.1(10)*	39.7 ± 10.4(10)†	

[a]mean ± SEM (number per group); *significantly different from 3-4 month old. $p < 0.05$; †significantly different from estrous cohort groups. $p < 0.05$

Discussion. The present results are consistent with earlier reports which observed that the old CE rat has elevated circulating prolactin levels and decreased LH response to ovariectomy.[113,144,161-163] While the preceding study showed that DA turnover rates were severely decreased in ME tissues from old CE compared to young ovariectomized Long-Evans rats, the present data indicate that hyperprolactinemia is not the result of impaired TIDA activity in old CE rats. Rather, prolactin levels appear to be maintained by ovarian factors (presumably estrogen) since castration reduced prolactin in CE rats to basal levels equivalent to levels in young ovariectomized rats. Since the magnitude of prolactin increase after αMPT administration was similar in all age groups, this further indicates that TIDA function remains sufficient in old rats to maintain basal prolactin secretion in the absence of gonadal steroids. Interestingly, the rate of prolactin increase after drug administration appeared slower in old versus young rats. Although this may suggest altered DA regulation of lactotroph secretion occurs in old Long-Evans rats, the present data clearly indicate that elevated prolactin results primarily from ovarian rather than hypothalamic or AP changes in the CE rat.

The mechanisms responsible for dampened LH response to ovariectomy in old CE rats remain unclear. The ability of repeated LHRH injections to elicit similar LH secretion profiles in both old CE and young rats indicates that gonadotrophs remain functional.[127] Results of the preceding studies showed that LHRH concentrations remained stable through advanced age but CA activities were decreased in several hypothalamic regions of old compared to young rats. Since disruption of NE activity has been shown to dampen LH secretion in young ovariectomized rats (see 49, 50, 68-72), impaired NE function may contribute to decreased LH response in old CE rats.

However, it is unlikely that dampened NE turnover alone can explain decreased LH response in old CE rats since NE turnover rates were also dampened in several hypothalamic regions from middle-aged rats which had relatively normal LH response to ovariectomy. This suggests that impaired NE neuronal function may precede dampened LHRH neurosecretion. Interestingly, LH tended to decrease after αMPT administration in both middle-age and old ovariectomized Long-Evans rats but was unchanged in young animals. The differential response of LH to CA synthesis inhibition between young and older animals may indicate that an alteration occurs during advancing age in CA regulation of LHRH neuron function. Whether these possible changes involve NE activity might be examined by testing the ability of adrenergic agonists to improve LH secretion in old CE animals.

In contrast to most previous studies in old CE rats, the present study showed that LH concentrations were increased in old CE rats compared to young animals killed on estrus morning.[114,148] Since the vast majority of studies have examined hormone levels from samples obtained in etherized animals,[114,144,148] prior stress may differentially decrease LH in old but not young animals. It was noted that old CE ovariectomized rats which received an i.p. saline injection had LH concentrations which were less than one half those found in rapidly decapitated CE rats in the present studies. One study which examined LH in samples collected at decapitation in CE Long-Evans rats showed LH may have been increased compared to proestrous rats killed during the morning;[220] however, data were not analyzed for these possible differences.

Age-Related Alterations in the Regulation of Pulsatile Luteinizing Hormone Release in Ovariectomized Constant Estrous Rats

Changes of Pulsatile Luteinizing Hormone Secretion Profiles in Ovariectomized Rats During Advanced Age

Objectives. The pulsatile nature of LH secretion appears to depend upon episodic secretion of LHRH into the portal vasculature.[28,81] Removal of the inhibitory effects of gonadal steroids, augments the magnitude of pulsatile LH secretion as reflected in hormone profiles obtained from ovariectomized rats.[81] An increasing body of evidence indicates that NE neurons facilitate LHRH neuronal function in the ovariectomized as well as the steroid treated rat (see 49, 50, 81). Results from the preceding study clearly showed that the old CE rat has a dampened LH response to ovariectomy, as has been reported by several other investigators.[113,144,161-163] The decreased NE neuronal activities noted in several hypothalamic regions from old CE ovariectomized rats compared to young animals suggests that LHRH neurons in these animals may not receive sufficient NE stimulation for optimal function. Although our study of LHRH concentrations and depletion response to ovariectomy revealed no significant alterations in the old CE rat, an altered mode of pulsatile LHRH release might explain the dampened LH secretion response to ovariectomy seen in these animals.[28,29] The present study was designed to determine if an age-related decrease in LH secretion is due to defects in the pulsatile patterns of LH release.

Materials and Methods. Female Sprague-Dawley (Charles Rivers) rats were purchased at 2-3 and 8-10 months of age and maintained in our animal facility. Daily vaginal lavages were examined for at least two weeks when rats were 2-3, 8-10 and 16-17 months of age to characterize the reproductive state of each animal. Rats were then ovariectomized and three weeks

later implanted with an atrial catheter via the jugular vein as described above. Two days following catheterization, 13 serial blood samples were obtained from freely moving rats at 15 min intervals over a 3 h (1230 - 1530 h) sampling period. Upon completion of blood sampling, animals were killed and necropsied. One 17-18 month old animal had a visible pituitary adenoma and data obtained from this animal were excluded from analysis.

Plasma was assayed in 25 and 100 µl volumes for LH concentration with standard RIA methods. Intraassay coefficient of variance was 15.6%. Remaining plasma samples from each individual animal were then pooled and prolactin concentrations were determined. Plasma prolactin values were expressed in terms of NIAMDD rat PRL-RP-1. Mean concentration of LH was determined by averaging all LH values from each animal. The amplitude of each LH pulse was determined as the height of the increasing phase of each pulse. The decreasing phase of the pulse represents clearance of LH in the absence of pituitary secretion[265] and, therefore, was not included in calculation. The significance of difference in pulse amplitude as well as mean plasma LH levels between groups was evaluated by one-way analysis of variance followed with least significant difference tests.

Results. Representative profiles of LH pulses for animals of each age and reproductive state used in this study are shown in Figure 5. Pulse amplitude in old CE rats was significantly lower than all other groups studied, while LH pulse amplitudes in 3-4 month NC rats were significantly greater than all other groups except 9-11 month NC rats (Table XVIII). There was no significant difference between 9-11 month NC and IC groups or between 9-11 month IC and 17-18 month IC animals.

Mean plasma LH concentration was decreased and plasma prolactin increased in 17-18 month rats when compared to younger animals (Table XIX). However,

Figure 5. Representative Luteinizing Hormone (LH) Secretion Profiles in Aging Sprague-Dawley Rats Ovariectomized at Various Reproductive States.

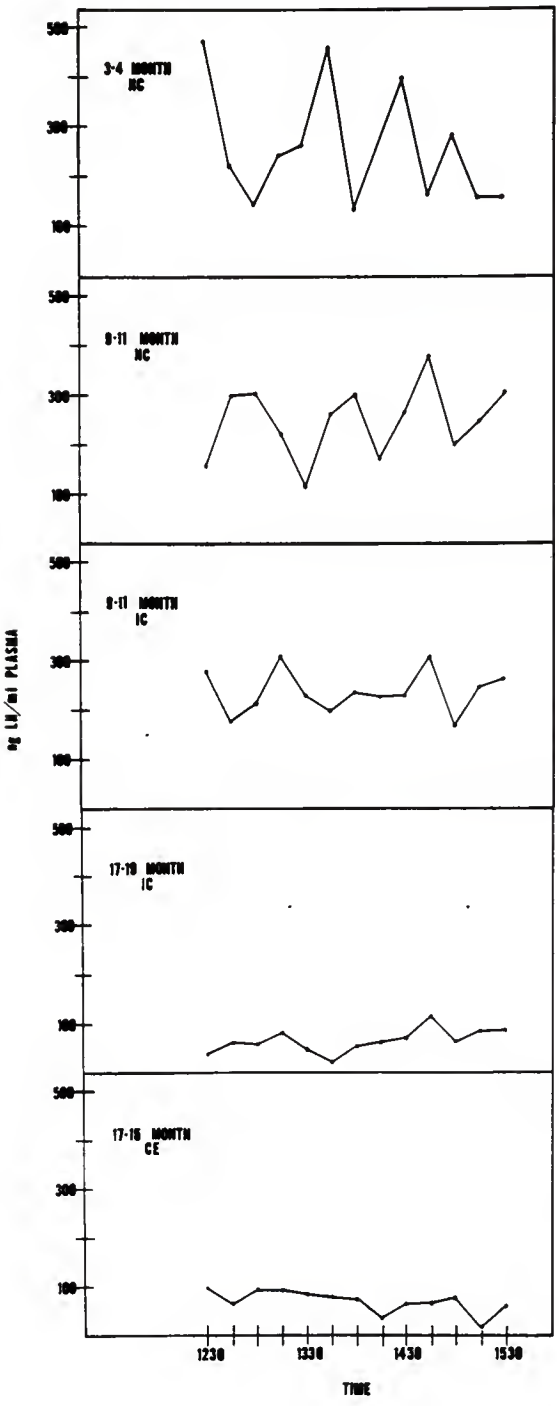
500
300
100
3-4 MONTH
NC
9-11 MONTH
NC
9-11 MONTH
IC
17-19 MONTH
IC
17-19 MONTH
CE
ng LH/ml PLASMA
1230
1330
1430
1530
TIME

TABLE XVIII. Luteinizing Hormone Pulse Amplitude in Ovariectomized Rats of Several Ages and Reproductive States

Age (months)	NC	IC	CE
	LH Pulse Amplitude (ng increase/ml plasma)		
3-4	163 ± 32(8)*,a	---	---
9-11	117 ± 16(13)a,b	86 ± 16(14)b	---
17-18	---	90 ± 25(10)b	37 ± 4(28)c

*mean ± SEM (number/group) Groups with the same superscript are not significantly different at $p < 0.05$

TABLE XIX. Age-Related Changes in Mean Plasma Luteinizing Hormone Concentrations in Ovariectomized Rats

Age (months)	Number of Rats	Prolactin (ng/ml)	LH (ng/ml)
3-4	10	$1.9 \pm 0.2^{a*}$	$263 \pm 16^{a*}$
9-11	7	$6.8 \pm 3.4^{a,b}$	193 ± 22^{a}
17-18	9	16.2 ± 4.3^{b}	85 ± 15^{b}

*mean ± SEM Groups in the same column (hormone) with the same superscripts are not significantly different at $p < 0.05$

regardless of age, there was no good correlation (r = .434) for individual animals between LH pulse amplitude and prolactin levels.

Discussion. This study establishes that the inability of old CE rats to show a normal postcastration LH increase is due to a relative absence of pulsatile LH release. Further, the decrease in amplitude of LH pulses appears to be closely associated with the progressive change in reproductive states which accompanies aging in the rat. Thus, the highest LH pulse amplitudes were observed in 3-4 and 9-11 month old NC rats and lowest amplitudes in 17-18 month old CE animals. Mean LH pulse amplitude for IC rats (both 9-11 and 17-18 month old groups) were between NC and CE values.

The observation that in the 9-11 and 17-18 month old groups, multiple reproductive states were represented, indicates that the rate of reproductive aging varies among animals. These data indicate that amplitude of pulsatile LH release is related more closely to state of reproductive function at the time of ovariectomy than to age of the animal. The relationship between pulse amplitude and reproductive state supports the concept that a central deficiency, progressive in nature and variable among rats of the same age, may be responsible for change in estrous cycles.

In the young ovariectomized rat, LH release appears to be a result of NE mediated pulsatile LHRH secretion from the hypothalamus (see 81). In view of the preceding results which showed partially impaired NE activity in several hypothalamic regions in old ovariectomized CE compared to young NC rats, the present results indicate that the response of LHRH neurons to these CA alterations may be severely impaired episodic function. The ability of old CE rats to secrete LH in patterns similar to young rats after repeated LHRH injections[127] further indicates that an altered LHRH release function, rather than an AP impairment, is primarily responsible for dampened episodic

LH secretion in old ovariectomized CE rats. While it has been demonstrated that diminished NE activity dampens LH release in young ovariectomized rats (see 81), whether augmenting NE function can restore LH release in old CE animals has not been shown. However, the ability of centrally acting stimuli to restore ovulatory function in these animals (see 105) suggests that impaired CA function is causally related to impaired LH secretory response.

Alternatively, high circulatory levels of prolactin, which inhibit LH secretion,[266] may be involved in dampening the amplitude of LH surges. In the present study, although an age-related elevation in plasma prolactin was observed, no close correlation ($r = .434$) between plasma prolactin and LH pulse amplitude was observed for individual animals. However, this relationship should be considered with caution since prolactin concentration reported here represents basal levels in ovariectomized rats.

Restoration of Pulsatile Luteinizing Hormone Release by Clonidine in Young Ovariectomized Rats with Acute Norepinephrine Depletion

Objectives. The pulsatile nature of LH secretion which is clearly reflected in LH profiles of ovariectomized rats appears to depend upon NE mediated intermittent release of LHRH (see 81). The observation that increased hypothalamic CA turnover was associated with augmented LH release in ovariectomized rats provided indirect evidence for a stimulatory role of CA on LHRH neurons.[59-63] Suppression of pulsatile LH release by adrenergic receptor blockers[265] or drugs which inhibited NE synthesis[68,69] further indicated the facilitory role of central adrenergic systems on LHRH release. Although these studies showed that adrenergic systems may modulate LH secretion, more direct evidence such as the restoration of pulsatile LH secretion by adrenergic agonists in NE depleted ovariectomized rats has not

been demonstrated. Furthermore, the precise mode of adrenergic involvement in maintenance of pulsatile LH release is not clearly understood.

The severely dampened pulsatile LH secretion profiles of old CE rats in response to ovariectomy which were shown in the preceding study closely resemble those observed in the young ovariectomized rat following blockade of NE synthesis.[69,81] Since dampened NE turnover rates were seen in several hypothalamic regions from old ovariectomized CE rats, a less expensive animal model was sought to more directly examine the dependence of LH pulsatile secretion on adrenergic stimulation. Therefore, relatively inexpensive ovariectomized young rats in which NE synthesis was pharmacologically blocked were selected to examine the ability of an adrenergic receptor agonist to restore pulsatile LH secretion.

Materials and Methods. Young mature Sprague-Dawley rats (Charles Rivers) were ovariectomized within a few days after arrival in our animal facility. Two weeks after ovariectomy, animals were fitted with indwelling atrial catheters via the jugular vein as described above. On the following day, serial blood samples were withdrawn at 10 to 15 min intervals to characterize the effects of drugs which alter adrenergic activity on pulsatile LH secretion profiles.

Rats in Experiment 1 were pretreated with a DBH inhibitor, diethyldithiocarbamate[267] (DDC, 550 mg/Kg, i.p.) and 1 h later blood samples were withdrawn at 15 min intervals. Immediately following the fifth blood sample rats were additionally treated with saline vehicle or 0.03 or 0.3 mg/Kg of the α-adrenergic agonist, clonidine[268] (CLON, Catapres, Boehringer Ingelheim Ltd.) via the cannula and blood sampling continued at 15 min intervals for an additional 2 h.

Rats in Experiment 2 were pretreated 1 h before blood sampling with another DBH inhibitor,[269] bis(4-methyl-1-homopiperanzynyl thiocarbonyl)-disulfide (FLA-63, 25 mg/Kg, i.p., in 0.1 N hydrochloric acid, Regis Chemical) or vehicle alone (untreated controls). Immediately following the fifth blood sample, these rats received additional treatment of 0.3 mg/Kg CLON or saline and blood samples were withdrawn at 15 min intervals for 2 h.

The third experiment was designed to determine the duration of CLON effectiveness. Two groups of rats were treated with FLA-63 as described above and blood samples were withdrawn at 15 min intervals for 1 h prior to additional treatment of CLON (0.3 mg/Kg) or saline. Blood sampling was begun 2 h after CLON or saline injection for a period of 2 h. The fourth experiment was designed to more carefully characterize the pattern of LH secretion following CLON administration. Three groups of rats were pre-treated with FLA-63 or saline and 1 h later, additionally treated with 0.3 mg/Kg CLON or saline. Blood samples were withdrawn at 10 min intervals for 3 h beginning immediately prior to CLON administration.

To assess the effect of DDC and FLA-63 on CA concentrations, NE and DA in the POA and MBH were determined at various intervals after administration of the DBH inhibitors. Ovariectomized rats treated with DDC were killed at 1 and 4 h; and rats treated with FLA-63 were killed at 1, 2 and 5 h after drug treatment. The POA and MBH were dissected immediately after decapitation and homogenized in 0.4 N perchloric acid for subsequent evaluation of DA and NE concentrations. The significance of differences between saline and drug treated groups was determined by Student's t-tests.

The effects of FLA-63 and DDC alone or of CLON injection in FLA-63 and DDC treated rats on pulsatile LH secretion was initially analyzed by

comparing the changes in mean LH pulse amplitude and the mean plasma LH concentrations before and after CLON or saline (control) administration. These experiments were conducted over a two year period, during which improved techniques were published to analyze pulsatile hormone secretion.[270,271] The different methods employed in the present studies to characterize pulsatile LH secretion therefore reflect the increasing emphasis on intraassay variability in these improved analytical techniques. For the first three experiments pulse amplitude was defined as the height of the increasing phase of each pulse and it represented the peak minus the preceding nadir LH values for the rising phase of the pulse. Pulse amplitudes which fell below 10 ng/ml were discarded since these differences were below the intraassay variability. Pulse amplitude values from rats in a group were pooled and presented as group mean pulse amplitude (ng LH/ml). Mean plasma concentrations from each treatment period represented the average of all values found in the group. Plasma LH concentrations were analyzed in 25 µl and 100 µl volumes for each sample with standard RIA methods. The average intraassay coefficient of variation (for experiments 1, 2 and 3) was 15.7% and was calculated from eight replicate samples of a standard pooled sera in volumes which resulted in approximately 50% inhibition of total specific binding. Since statistical comparison between experiments was not attempted, interassay variation was not calculated. The significance of differences in both mean pulse amplitude and mean plasma LH concentrations before and following injection of test material was evaluated by Student's t-test.

Experiment four was designed to evaluate both pulse amplitude and interpulse interval. Thus, a more rigorous analysis was employed to define and evaluate LH pulses. Intraassay coefficient of variation was 5.9% and 16.8% for pooled sera with mean LH concentrations of 361 and 162 ng/ml

respectively, when assayed in 10 replicates each. The further evaluation of LH pulse used the method described by Gallo.[270] Briefly, an animal was determined to exhibit pulsatile LH secretion if the coefficient of variation of LH concentration for all samples obtained from that animal was greater than 1.5 times the intraassay coefficient of variation. Individual LH profiles were then graphed and the coefficient of variation was determined for each suspected LH pulse, utilizing LH concentrations on both the ascending and descending portions of the pulse. If this coefficient of variation was greater than 1.5 times the appropriate intraassay coefficient of variation, the pulse was utilized in further calculations. Interpulse interval was defined as the time between successive peak LH levels for identified LH pulses. Luteinizing hormone pulse amplitude was determined as described above. Group differences for LH pulse amplitude and interpulse interval were evaluated by analysis of variance and least significant difference tests with $p < 0.05$ chosen as the level of significance.

Results. In the DDC pretreated rats, serum LH levels during the 2 h experimental period after saline injection fluctuated little and the pattern was similar to that observed during the control 1 h presaline injection period (Table XX and Figure 6). However, CLON administration altered the LH secretion pattern, which was characterized by abrupt stimulation of LH release peaking within 15-30 min followed by episodic LH fluctuations. The dose of 0.3 mg/kg was more effective than 0.03 mg/kg dose because it uniformly stimulated episodic LH secretion in experimental rats. In these rats, both mean serum LH concentration and mean pulse amplitude during post-CLON-period were significantly higher than those observed in the pre-CLON control period. On the other hand, 0.03 mg/kg CLON stimulated episodic

TABLE XX. Effects of Various Doses of Clonidine (CLON) on Pulsatile Luteinizing Hormone Release in Ovariectomized Rats Pretreated with Diethyldithiocarbamate (DDC)

Pretreatment	Additional Treatment	Number of Rats	Mean LH Pulse Amplitude[a] (ng/ml)		Mean Plasma LH[a] (ng/ml)	
			Preadditional Treatment Period	Postadditional Treatment Period	Preadditional Treatment Period	Postadditional Treatment Period
DDC	Saline	7	35 ± 7(9)[b]	55 ± 10(18)	92 ± 5(35)[c]	95 ± 5(55)
DDC	CLON (0.03 mg/kg)	5	56 ± 11(6)	113 ± 19(9)*	126 ± 11(25)	136 ± 11(40)
DDC	CLON (0.3 mg/kg)	5	65 ± 17(8)	138 ± 21(15)*	172 ± 13(25)	228 ± 13(40)*

[a]See Materials and Methods for explanation for determination of mean pulse amplitude and mean plasma concentration of LH levels; [b]mean ± SEM (pulses/group); [c]mean ± SEM (plasma samples/group); *LH response significantly different from preadditional treatment period control value. $p < 0.05$

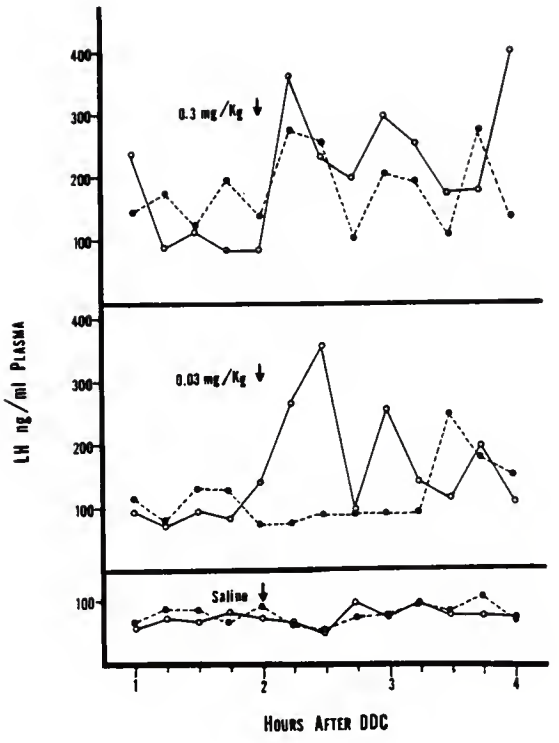

Figure 6. Representative Luteinizing Hormone (LH) Secretion Profiles of the Effects of Clonidine (CLON, 0.03 and 0.3 mg/Kg) and Saline in Ovariectomized Rats Pretreated with Diethyldithiocarbamate (DDC). Animals were pretreated with DDC 1 h prior to the onset of blood sampling and CLON or saline was administered 1 h later.

LH release in three of five animals and the mean LH pulse amplitude alone was significantly raised by this dose of CLON (this suggested the frequency of LH secretion was decreased after DCC treatment). Despite the effectiveness of CLON (0.3 mg/kg) to stimulate pulsatile LH secretion in DDC treated rats, we discontinued the use of DDC in subsequent experiments because it failed to uniformly reduce plasma LH concentrations and LH pulse amplitude in the control period in the CLON treated rats to the level seen in control group (Table XX). Luteinizing hormone concentrations, for example, during the pre-CLON period of 0.3 mg/kg CLON group were higher than those observed during the same control period in saline treated group.

Suppression of episodic LH release following FLA-63 was more uniform than that seen after DDC treatment. Results shown on Table XXI show plasma LH levels and the mean LH pulse amplitude during the 1 h control period of rats receiving either saline (group B) or CLON (group C) was similar and significantly lower than seen in control rats receiving no drug pretreatment ($p < 0.05$, group A). Clonidine dramatically stimulated LH release in six of seven rats (group C and Figure 7). The mean LH pulse amplitude rose more than 4-fold and mean LH concentrations doubled in 2 h period after CLON treatment; this LH response was comparable to that found in control rats (group A). Surprisingly, the stimulatory effects of a single injection of CLON on LH release lasted longer than 2 h (Table XXII and Figure 7). While plasma LH levels continued to be suppressed between 2-4 h after saline treatment in FLA-63 treated rats, episodic LH secretion persisted in CLON treated rats during this 2 h period. The pattern of LH secretion during the 2 h period either immediately after (Table XXI, group C and Figure 7) or 2 h following CLON (Table XXII and Figure 7) injection was quite similar to that seen in control rats (Table XX, group A).

TABLE XXI. Luteinizing Hormone Release for Two Hours After Clonidine (CLON) Injection in Ovariectomized Rats Pretreated with FLA-63

Group	Pretreatment	Additional Treatment	Number of Rats	Mean LH Pulse Amplitude[a] (ng/ml)		Mean Plasma LH[a] (ng/ml)	
				Preadditional Treatment Period	Postadditional Treatment Period	Preadditional Treatment Period	Postadditional Treatment Period
A	Saline (Control)	Saline	7	106 ± 22(8)[b]	119 ± 19(14)	179 ± 18(35)[c]	184 ± 13(56)
B	FLA-63	Saline	7	47 ± 11(7)	54 ± 7(15)	119 ± 7(34)	117 ± 6(56)
C	FLA-63	CLON (0.3 mg/kg)	7	30 ± 7(10)	136 ± 24(21)*	107 ± 9(33)	241 ± 15(56)*

[a]See Materials and Methods for explanation for determination of mean pulse amplitude and mean plasma concentration of LH levels; [b]Mean ± SEM (pulses/group); [c]mean ± SEM (plasma samples/group); *LH response significantly from preadditional treatment period values. $p < 0.01$

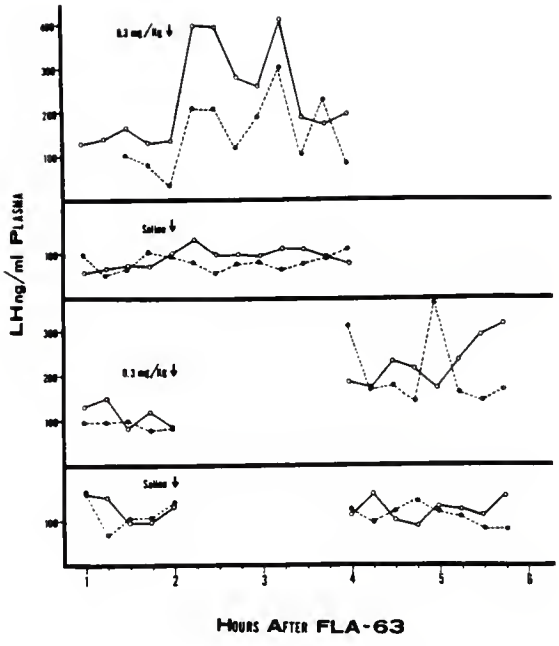

Figure 7. Representative Luteinizing Hormone (LH) Secretion Profiles of the Effects of Clonidine (CLON, 0.3 mg/Kg) or Saline in Ovariectomized Rats Pretreated with FLA-63. Rats were pretreated with FLA-63 1 h prior to the onset of blood sampling and CLON or saline was administered 1 h later. The upper two panels show the immediate effects of CLON (saline) while the bottom two panels show the effects of CLON (saline) 2-4 h after its administration.

TABLE XXII. Luteinizing Hormone Release Between Two-Four Hours After Clonidine (CLON) Administration in Ovariectomized Rats Pretreated with FLA-63

Pretreatment	Additional Treatment	Number of Rats	Mean LH Pulse Amplitude[a] (ng/ml)		Mean Plasma LH[a] (ng/ml)	
			Preadditional Treatment Period	Postadditional Treatment Period	Preadditional Treatment Period	Postadditional Treatment Period
FLA-63	Saline	7	43 ± 9(8)[b]	57 ± 9(17)	112 ± 7(35)[c]	132 ± 7(56)
FLA-63	CLON (0.3 mg/kg)	10	41 ± 5(13)	76 ± 12(22)*	121 ± 5(50)	235 ± 9(80)*

[a]See Materials and Methods for explanation for determination of mean pulse amplitude and mean plasma concentration of LH levels; [b]mean ± SEM (pulses/group); [c]mean ± SEM (plasma samples/group); *LH response significantly different from preadditional treatment value. $p < 0.05$

Treatment with FLA-63 decreased both LH pulse amplitude and mean LH levels by 52% and increased interpulse interval by 47% (Table XXIII, groups B vs. A). Treatment with CLON (group C) partially restored LH pulse amplitude and mean LH levels to those observed in ovariectomized control rats. Further, CLON treatment decreased interpulse interval to the level observed in ovariectomized control animals. The method utilized to define pulse in this experiment eliminated seven suspected pulses of low amplitude and as an apparent result LH pulse amplitude in FLA-63 treated rats was elevated when compared to similarly treated animals examined in experiment two (Table XXII, group B).

As shown on Table XXIV, treatment with DDC reduced the MBH, POA and NE concentration within 1 h. These low levels were still present at 4 h, when the CLON experiments were terminated. Similarly, FLA-63 treatment significantly suppressed the MBH and POA levels through 1-5 h after FLA-63 treatment. On the other hand, each of these drugs concurrently and significantly raised the DA levels in the MBH and POA.

Discussion. Administration of FLA-63 or DDC produced suppression of pulsatile LH secretion in ovariectomized rats within 1 h of treatment. Concentrations of NE were decreased simultaneously with significant elevations in DA levels in the MBH and POA of these rats. Although administration of DA agonists such as apomorphine have been shown to transiently suppress LH release in ovariectomized rats[68,69] the role of DA systems in episodic LH secretion has been questioned.[81] In our studies the decrease in LH secretion observed in FLA-63 and DDC treated rats cannot be attributed to elevations in the hypothalamic DA levels because, (i) the rise in DA concentration probably represents an accumulation in the NE projections following inhibition of DBH activity, and thus these levels may not represent

TABLE XXIII. Characterization of Luteinizing Hormone Release for Three Hours After Clonidine (CLON) Administration in Ovariectomized Rats Pretreated with FLA-63

Group	Pretreatment	Additional Treatment	Number of Rats	Mean LH Pulse Amplitude[a] (ng/ml)	Mean Plasma LH[a] (ng/ml)	Interpulse Interval[a] (min)
A	Saline	Saline	7	250 ± 30[b] (29)	323 ± 20[c] (113)	34 ± 3[b] (22)
B	FLA-63	Saline	6	119 ± 17* (16)	156 ± 7* (86)	50 ± 6* (10)
C	FLA-63	CLON	7	185 ± 22 (26)	257 ± 14** (93)	34 ± 3 (19)

[a]See Materials and Methods, Experiment 1d for explanation of determination of mean pulse amplitude, mean plasma concentration of LH levels and interpulse interval. [b]mean ± SEM (pulses or intervals/group); [c]mean ± SEM (plasma samples/group) *significantly different from group A, $p < 0.05$; **significantly different from group A and B, $p < 0.05$

TABLE XXIV. Effects of Diethyldithiocarbamate (DDC) and FLA-63 on Catecholamine Concentrations in the Preoptic Area and Medial Basal Hypothalamus

Treatment	Time after Treatment	Number of Rats	Catecholamine Concentration (ng/g Tissue)			
			MBH		POA	
			NE	DA	NE	DA
Saline	1 h	5	1207 ± 142[a]	433 ± 52	1020 ± 117	136 ± 12
DDC	4 h	6	407 ± 14*	506 ± 22	610 ± 63*	300 ± 21*
Saline	1 h	5	854 ± 109	351 ± 32	748 ± 34	131 ± 6
DDC		6	370 ± 38*	574 ± 48*	437 ± 70*	424 ± 30*
Saline	1 h	6	1118 ± 65	345 ± 29	1178 ± 60	185 ± 9
FLA-63		7	937 ± 88	692 ± 27*	809 ± 88*	335 ± 27*
Saline	2 h	6	1037 ± 41	276 ± 26	893 ± 67	140 ± 5
FLA-63		6	521 ± 19*	384 ± 34*	603 ± 26*	335 ± 6*
Saline	5 h	6	1212 ± 128	390 ± 25	1267 ± 38	158 ± 12
FLA-63		7	480 ± 78*	657 ± 48*	480 ± 74*	397 ± 27*

[a]Mean ± SEM; *significantly different from saline controls $p < 0.05$

accelerated neural activity in the hypothalamic DA systems, and (ii) the specific adrenergic agonist, CLON ,stimulated LH release.

Perhaps the most significant observation of these studies is that a single injection of CLON enhanced both the amplitude and frequency of LH pulses, an effect which persisted for as long as 4 h. Profiles of LH secretion stimulated by CLON were similar to those normally observed in ovariectomized rats. This response was due presumably to resumption of episodic LHRH discharge into the hypophyseal portal veins.[29,81] These results provide the first direct evidence for an excitatory role of α-adrenergic systems in augmenting and maintaining the pulsatile LH secretion in rats with acute brain NE deficiency.

The mechanisms whereby an injection of CLON produced a sustained pulsatile LH secretion in the FLA-63 and DDC treated rats are not understood. It is possible that the acute deficiency in the central adrenergic tone in these rats suppressed those neural mechanisms, probably resident within the LHRH neurons, which trigger the episodic neurohormone secretion. A bolus injection of CLON was apparently adequate to reactivate these processes and enhance episodic LH secretion. Thus, if a brief adrenergic activation can produce a sustained episodic LH secretion it would seem that in ovariectomized rats pulsatile LHRH release may not be tightly coupled with or be driven by regular episodic α-adrenergic stimulation.

Clonidine has been detected in the systemic circulation and in the rat brain for as long as 240 min following intravenous administration in doses ranging from 0.2-0.5 mg/Kg.[272] It is likely this long sojourn in the blood and/or by binding to the central adrenergic receptors for extended periods, CLON provided continuous excitatory stimuli, which led to hypothalamic activation of the pituitary at regular intervals in the FLA-63

and DDC treated rats. Along the same line, an alternate possibility would be that adrenergic receptor stimulation by CLON for extended periods merely restored a necessary permissive environment in the hypothalamus for episodic discharge of LHRH.

Thus, although we cannot fully discount the possibility that secretion of each pulse of LH (or LHRH) is a consequence of preceding adrenergic excitation, our observations that one injection of CLON can produce sustained episodic LH release are consistent with the view that central adrenergic systems may provide a permissive environment to optimally allow LH secretion to occur in a pulsatile fashion.

Restoration of Pulsatile Luteinizing Hormone Release by Clonidine in Aged Ovariectomized Long-Evans Rats

Objectives. The ability of clonidine (CLON) to restore pulsatile LH secretion in young ovariectomized rats that had impaired NE neuronal function, demonstrated in the preceding study, provided an experimental approach to directly test the ability of adrenergic stimulation to restore LH secretion in the old CE rat. Although other investigators have suggested a CA etiology for impaired LH secretion in old CE rats,[113,182] supporting evidence for this concept has been correlative or indirect. Some of this correlative evidence was shown in our studies of regional alterations in CA concentration and metabolism in aging CE rats. Further, the ability of several CA mediated stimuli to restore estrous cycles in old CE rats (see 105) does not clearly demonstrate normal preovulatory LH hypersecretion since as little as 15% of the normal LH surge can induce ovulation.[56]

The present study sought to identify the nature of the alteration in LH secretion in CE rats by characterizing the parameters of pulsatile LH release in animals two weeks after ovariectomy. The ability of α-adrenergic

stimulation to restore both amplitude and frequency of pulsatile LH secretion was then tested in these CE rats.

Materials and Methods. Young (4-5 month old) with regular four or five day estrous cycles, CE middle-aged (11-12 month old) and CE aged (21-23 month old) Long-Evans rats were selected for the present experiments. Middle-aged and old rats had been in the CE state for 2-3 and 6-10 months, respectively, at the initiation of these studies. Animals were ovariectomized and 13 days later implanted with atrial catheters via the jugular vein as described above. The following day serial blood samples were withdrawn at 10 min intervals for the 170 min experimental period. Immediately after withdrawal of each 0.5 ml blood sample, plasma was separated and frozen for assay of LH; cells were resuspended in heparinized saline and diluted cells were returned via the cannula to each rat after the subsequent blood sample.

To evaluate the effects of CLON on pulsatile LH secretion in these animals, additional animals from the same cohorts and with the same cycle characteristics were ovariectomized and cannulated as described above. Blood samples were obtained every 10 min for 1 h to establish a basal LH secretion profile for each animal. Following the sixth blood sample, CLON (0.3 mg/kg) was administered via the catheter and blood sampling resumed for an additional 2 h. No obvious behavioral effects of this dose of CLON were observed in middle-aged or old rats used in this study. Animals were necropsied at the end of the experimental period.

Plasma LH concentrations were evaluated in 100 µl samples by standard RIA methods as described above. The sensitivity of the LH assay was 2.5 ng/assay tube (25 ng/ml plasma). All LH plasma concentrations were determined in one assay.

The pulsatile nature of LH secretion was evaluated by a slight modification of the method described by Gallo.[270,271] Briefly, the coefficient of variation (CV) of LH concentrations was determined for individual animals and then for each treatment group. To determine if the variation in plasma LH samples for each group of rats was significantly greater than the assay variation, the CV for the LH secretory profiles was compared with the appropriate intraassay CV using unpaired Student's t-tests. Appropriate intraassay CV levels were selected as those which most closely matched mean LH levels for each animal. The intraassay CVs were determined by assaying 10 replicates of each of six standard pools of plasma which had mean LH concentrations of 37, 73, 124, 152, 221 and 285 ng/ml plasma. These pooled plasma samples were obtained from a single stock pool of male rat plasma to which increasing amounts of rat LH RP-1 standard were added. Table XXV presents the intraassay at these six mean LH concentrations and the corresponding ranges of sample plasma LH levels which were compared at these values. Thus, for a particular LH level, intraassay CV for that LH range was employed for statistical evaluation.

For individual LH secretory episodes, the mean hormone level and CV were calculated using LH values on both the ascending and descending portions of the suspected LH pulse. An LH pulse was considered significant when its CV was at least 2.0 times the appropriate intraassay CV (Table XXV). This criterion for significant LH pulses has been employed to evaluate the pulsatile LH secretory pattern during the estrous cycle,[271] the plateau phase of the proestrous LH surge[270] and the episodic LH secretion in ovariectomized rats.[81]

The parameters used to characterize LH secretion were mean LH levels, LH pulse amplitude (peak minus preceding nadir of each identified pulse)

TABLE XXV. Parameters of the Luteinizing Hormone Assay Employed in Evaluation of Pulsatile LH Secretion

Assay Standards ng LH/ml Plasma	Coefficient of Variation (CV,%)[b]	Corresponding Sample Range (ng LH/ml Plasma)[c]	Minimum Sample CV for LH Pulse[d]
37 ± 6[a]	16	< 55	32
73 ± 20	27	56 - 99	54
124 ± 30	24	100 - 139	48
152 ± 23	15	140 - 187	30
221 ± 33	15	188 - 253	30
285 ± 31	11	> 253	22

[a]mean ± standard deviation (n=10); [b]CV = mean LH/standard deviation x 100; [c]mean LH concentrations from an individual animal or the nadir LH level of a suspected pulse were assigned to one of these six ranges for comparison with appropriate assay CV; [d]suspected pulse CV's which exceeded these corresponding assay values were identified as significant pulses

and LH pulse frequency (number of identified pulses per h). This latter estimate of pulse frequency was chosen over the more routinely used estimate of interpulse interval since older animals often had only one or no LH pulse during the experimental period.

These three LH pulse parameters were statistically analyzed with one-way analysis of variance followed by Least Significant Difference (LSD) tests. The first experiment compared each parameter between age groups while the second experiment compared LH parameters before (pre-CLON) and after (post-CLON) CLON treatment within age groups. Plasma prolactin concentrations were measured in the first sample obtained from each animal using standard RIA methods.

Results. Table XXVI shows results obtained at necropsy. Body weights were increased significantly in middle aged (11%) and old (23%) rats compared with young animals. Age-related increases in anterior pituitary, kidney, and heart weights were also significant. However, no changes were observed with age when these organ weights were analyzed as a proportion of body weight. Adrenal weights were not significantly different between age groups.

No visible internal abnormalities were observed in young and middle aged rats at necropsy. Five of the old animals (31%) had small unilateral anterior pituitary hemorrhagic lesions. When anterior pituitary weights, body weight ratios, and plasma PRL concentrations from these lesioned rats were compared to nonlesioned old rats, no differences were detected. Two 21-23 month old rats which had anterior pituitary adenomas were excluded from the study. Anterior pituitary weights in these rats were 90 and 200 mg; plasma PRL levels exceeded 2000 ng/ml, and plasma LH was undetectable.

TABLE XXVI. Body and Organ Weights of Ovariectomized Long-Evans Rats Employed in Study of Clonidine Effects on Pulsatile Luteinizing Hormone Secretion

		Young	Middle-aged	Old
Body Weight (B.W., g)		296 ± 9	329 ± 9*	365 ± 10*†
Anterior Pituitary	Weight (mg)	12.0 ± 0.4	12.8 ± 0.3	15.3 ± 1.0*†
	mg/100 g B.W.	4.2 ± 0.3	3.9 ± 0.1	4.1 ± 0.2
Adrenal	Weight (mg)	79.3 ± 3.4	80.6 ± 2.8	86.6 ± 3.4
	mg/100 g B.W.	23.3 ± 0.8	24.9 ± 1.3	27.2 ± 1.1
Kidney	Weight (g)	1.93 ± 0.04	2.27 ± 0.04*	2.76 ± 0.10*†
	g/100 g B.W.	0.74 ± 0.03	0.69 ± 0.02	0.66 ± 0.02
Heart	Weight (g)	0.91 ± 0.03	0.93 ± 0.02	1.25 ± 0.04*†
	g/100 g B.W.	0.34 ± 0.01	0.29 ± 0.01	0.31 ± 0.01

*$p < 0.05$ versus young group; †$p < 0.05$ versus middle-aged group

All young rats exhibited pulsatile LH secretion 14 days after ovariectomy as shown on Figure 8A. The three analyzed secretory paramaters in 4-5 month old rats were: mean LH concentration, 137 ± 8 ng/ml plasma; LH pulse amplitude, 154 ± 12 ng/ml plasma; and LH pulse frequency, 1.16 ± 0.13 pulses/h. In marked contrast only four of seven middle-aged and two of six old ovariectomized rats exhibited significant pulsatile LH secretion (Table XXVII, Figure 8B and C). Of these six animals with pulsatile LH profiles, four had a single LH secretory episode and the remaining two showed two episodes during the 3 h period. Mean LH concentrations were decreased 67% and 70% in middle-aged and old rats, respectively, compared to young animals. This decrease appared to result primarily from a decline in pulse frequency of 78% in middle-aged and 84% in old rats, although pulse amplitudes also tended to be dampened in older animals.

Profiles of LH from middle-aged and old rats were dampened during the 1 h pre-CLON period (Table XXVIII) and were similar to those obtained over the 3 h period in the first experiment (Table XXVII). In response to CLON, nine of nine middle-aged rats showed pulsatile LH secretion (Figure 9). In these rats, CLON caused a 2-fold increase in mean LH levels, a 2-fold increase in LH pulse amplitude and nearly a 3-fold increase in LH pulse frequency (Figure 9, Table XXVIII). Treatment with CLON resulted in pulsatile LH release in three of eight old rats (Figure 10), although these secretory episodes were infrequent (0.37 ± 0.21 pulses/hr) and only modestly increased mean LH levels (35%). The amplitude of the seven LH pulses which were defined during the post-CLON period (Table XXVIII) was not significantly different from the LH secretory episodes observed during the 3 h sampling period in non-CLON-treated old ovariectomized rats (Table XXVII).

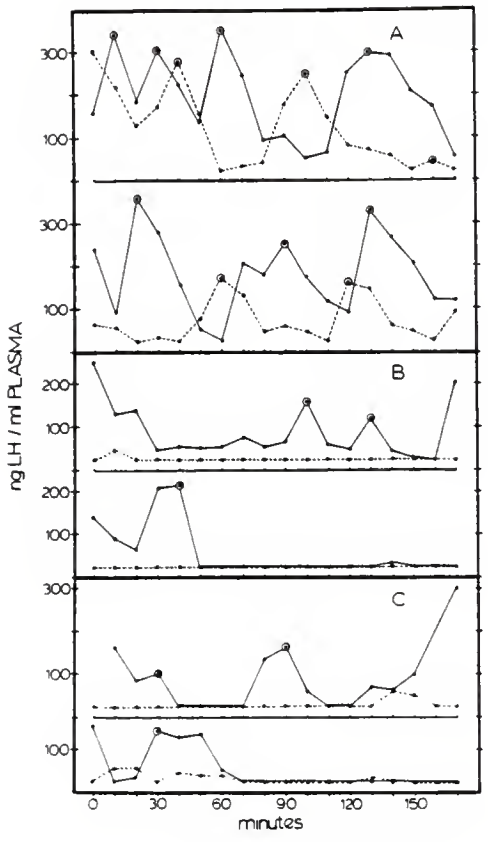

Figure 8. Representative Luteinizing Hormone (LH) Secretion Profiles in Ovariectomized Long-Evans Rats of Three Ages. Panels A, B and C each illustrate 4 individual profiles from young (4-5 month old), middle-aged (11-12 month old) and old (21-23 month old) rats, respectively. Large circles indicate significant LH pulse episodes as defined by criteria discussed in Materials and Methods.

TABLE XXVII. Age-Related Changes in Luteinizing Hormone Secretory Parameters of Ovariectomized Long-Evans Rats

Age (months)	Number of Rats	Mean LH (ng/ml Plasma)	LH Pulse Amplitude (ng/ml Plasma)[b]	LH Pulse Frequency (pulses/rat/h)
4 - 5	7	137 ± 8(126)[a]	154 ± 12(23)	1.16 ± 0.13
11 - 12	7	45 ± 4(126)**	96 ± 19(5)*	0.25 ± 0.10*
21 - 23	6	40 ± 4(107)**	85 ± 37(3)	0.18 ± 0.12*

[a]mean ± SEM (number of samples or pulses included in statistics);
[b]only statistically significant pulses were evaluated. See Material and Methods for pulse identification criteria; *$p < 0.05$ versus 4-5 month old group; **$p < 0.01$ versus 4-5 month old group

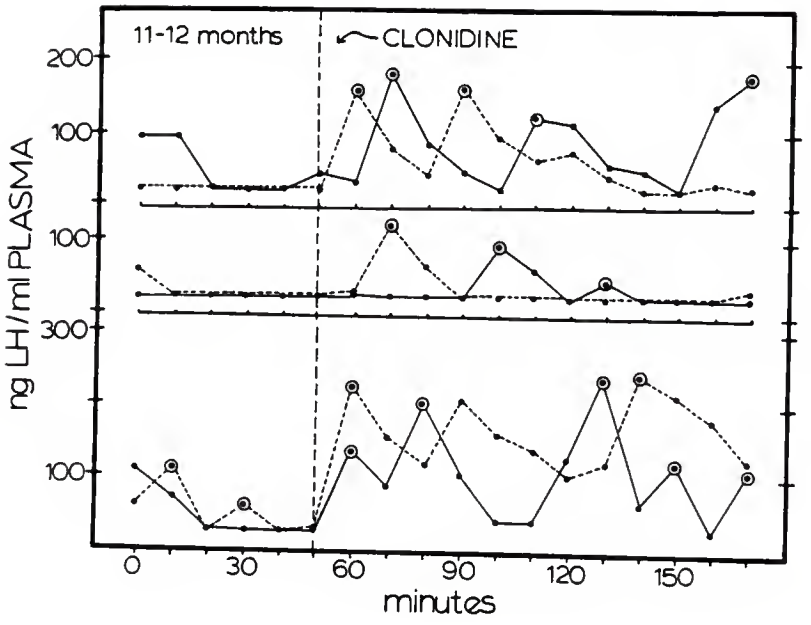

Figure 9. Representative Luteinizing Hormone (LH) Secretion Profiles for Six of Nine Individual 11-12 Month Old Ovariectomized Long-Evans Rats Treated with Clonidine (CLON). Clonidine (0.3 mg/Kg, iv) was administered immediately following the sixth blood sample. Large circles indicate significant LH pulse episodes as defined by criteria discussed in Materials and Methods

TABLE XXVIII. Effects of Clonidine on Luteinizing Hormone Secretory Parameters in Ovariectomized Middle-Aged and Old Long-Evans Rats

Age (months)	Number of rats	Treatment Period	Mean LH (ng/ml Plasma)	LH Pulse Amplitude (ng/ml Plasma)[b]	LH Pulse Frequency (pulses/rat/h)
11-12	9	PreCLON	42 ± 4(54)[a]	65 ± 13(3)[a]	0.33 ± 0.17
		PostCLON	90 ± 6(108)**	124.6 ± 10.4(18)*	0.89 ± 0.18*
21-23	8	PreCLON	32 ± 3(48)	ND	ND
		PostCLON	49 ± 5(95)*†	90 ± 21(6)	0.37 ± 0.21

[a]Mean ± SEM (number of samples or pulses included in statistics); [b]only statistically significant pulses were evaluated. See Material and Methods for pulse identification criteria; *$p < 0.05$ versus corresponding PreCLON value; **$p < 0.001$ versus corresponding PreCLON value; †$p < 0.001$ versus 11-12 month old PostCLON value; ND Parameter could not be determined as no pulses were significant

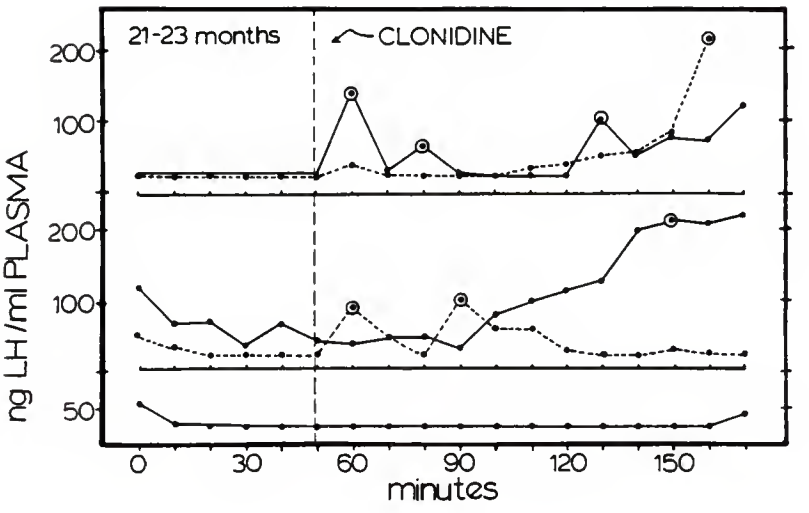

Figure 10. Representative Luteinizing Hormone (LH) Secretion Profiles for Five of Eight Individual 21-23 Month Old Ovariectomized Long-Evans Rats Treated with Clonidine (CLON). The top 2 panels illustrate profiles in the 4 rats which had significant LH pulses (large circles) following CLON (0.3 mg/Kg). The bottom panel is representative of the 4 animals which had persistent minimally detectable LH concentrations during the experimental period.

Discussion. A major finding of this study is that the diminished postcastration hypersecretion of LH in middle-aged and old CE Long-Evans rats appears to result from a substantial decline in the frequency and, to a lesser extent, the amplitude of LH pulses. The initiation of normal pulsatile LH secretion typical of young ovariectomized rats following a single dose of CLON in middle aged but not old rats indicates that the initial loss of pulsatile LH secretion involves a deficiency in the central adrenergic system. With increasing age and/or length of time in the CE state, deficiencies secondary to a decline in NE activity may render the hypothalamo-hypophyseal-LH secretory mechanism refractory to centrally-mediated stimuli for LH secretion.

A severely diminished LH secretory response to ovariectomy in the CE rat has been described by several investigators.[161-163] In the rat, the episodic discharge of this gonadotropin is initiated by pulsatile release of LHRH from the basal hypothalamus.[28,29,81] The present study indicates that with the onset of the CE state, the frequency of LHRH signals from the hypothalamus decreases to such an extent that hypersecretion of LH does not occur. The decline in the amplitude of LH pulses in older animals may further contribute to the diminished postcastration hypersecretion of LH in these rats.

While the age-related decline in LH pulse frequency clearly indicates a decline in the rate of discharge of LHRH from the MBH, dampened LH pulse amplitude may be due to CNS and/or gonadotroph alterations. The magnitude of LH response to a single dose of LHRH is dampened in old versus young rats;[127] however, multiple treatment with LHRH results in LH levels which are similar in both old and young rats.[127] Thus, gonadotrophs are able to respond relatively normally in CE rats. Although the

results of the previous study observed no effects of age on LHRH response to ovariectomy, dampened LHRH secretion modes may contribute to decreased pulse amplitude in ovariectomized, previously, CE rats.

In the middle-aged rat, the decline in LH pulse frequency and amplitude is rectified by CLON administration such that the LH secretory pattern following CLON treatment of middle-aged rats is quite similar to that observed in young controls. These observations are quite consistent with the hypothesis that a central adrenergic deficiency mediates the decreased LH secretory capacity of middle-aged CE rats.[264]

CLON was considerably less effective in inducing normal LH secretory patterns in old compared to middle-aged rats. Only two of eight old animals exhibited repeated LH pulses following CLON treatment. Since the amplitude of the six identified LH pulses was not significantly different, but the pulse frequency was only one-third of those observed in middle-aged rats after CLON treatment, α-adrenergic stimulation was apparently ineffective in enhancing the frequency of LHRH discharges from the hypothalamus in these old rats. Whether this decline in responsiveness to CLON in old rats reflects a decreased number and/or sensitivity of hypothalamic adrenergic receptors, as has frequently been described for extrahypothalamic adrenergic receptors in old rats,[199,228-231] or simply an alteration in the release patterns of LHRH is at present uncertain. The occurrence of small hemorrhages in the anterior pituitaries of 31% of old rats was not sufficient to account for the decreased response to CLON since (i) there was no difference in response between rats with or without hemorrhages, and (ii) the old rat which exhibited the most marked secretory response to CLON was observed to have a small pituitary hemorrhage. Rather,

the data indicate that with persistence of the CE state, a decline in the LH secretory response to α-adrenergic stimulation ensues.

The present results are consistent with the hypothesis that the neuroendocrine events which initiate the CE state may differ from those which maintain this noncyclic condition. A decline in noradrenergic activity appears to precede and participate in the onset of the CE state.[264] Thus, α-adrenergic stimulation soon after the onset of the CE state results in a uniform reinitiation of pulsatile LH secretion. With persistence of the CE state, further alteration in the central adrenergic system may appear as evidenced by the relative inability of CLON to augment pulsatile LH secretion in these old rats. Thus, the high variability reported in several studies of the response of CE rats to drug regimens which were intended to reinitiate estrous cycles (see 105) may simply reflect varying lengths of time in the CE state prior to drug intervention. Lu et al.[149] have suggested that chronic exposure to estrogen during constant estrus diminishes the LH secretory response to gonadal steroids. Our present observation that the LH response to adrenergic stimulation is diminished with increasing length of the CE state may reflect the effects of prolonged estrogen exposure. Further efforts to elucidate the neuroendocrine alterations underlying reproductive senescence should consider the length of the acyclic condition as an independent variable.

Reinitiation of Estrous Cycles in Old Pseudopregnant Rats with a Dopamine Agonist

Objectives. Results of the preceding studies strongly indicate that impaired NE function contributes to the onset of the CE state in aging Long-Evans rats. However, neither LH secretion response to ovariectomy or LHRH neuronal function appear severely altered in old PP F344 rats.

Unlike CE rats, PP rats maintain hyperprolactinemia in the absence of ovarian steroids.[159 161 175] Since old PP rats do ovulate periodically, augmented prolactin levels may function to maintain corpora lutea and contribute to maintenance of the PP state in these animals.

This study was designed to reduce serum prolactin with a DA agonist and monitor effects of this drug on estrous cycles in old F344 rats which had displayed the PP state for several months.

Materials and Methods. To evaluate the role of prolactin in the maintenance of the PP state of F344 rats, 14 age-matched PP rats (24-31 month old) were treated daily for 10 days between 1400 and 1500 h with the dopamine agonist, CB-154 (3 mg/kg, s.c., Sandoz Co., Hanover, NJ) or 60% ethanol vehicle. All animals had been in the PP state for at least three months prior to their use in this experiment. Daily vaginal lavages were monitored for 10 days prior to and 45 days after initiation of treatment. To avoid the complexing effects of stress on ovarian cyclicity in old rats,[132] no other invasive procedures were performed during this experiment. This dose schedule of CB-154 was chosen on the basis of its capacity to suppress PRL secretion.[273,274]

Results. Daily treatment of 24-31 month old F344 rats which had been PP for at least three months with the dopamine agonist, CB-154, reinitiated estrous cycles in seven of seven rats (Figure 11). All drug-treated rats exhibited proestrus vaginal smears within two or three days of the first CB-154 injection and had two normal four to five day cycles during the 10 day treatment period. Interestingly, two to four near normal cycles were maintained in five of the seven rats after cessation of CB-154 treatment. One rat had an abnormal cycle with five consecutive days of estrous smear types and the remaining rat returned to diestrus immediately after

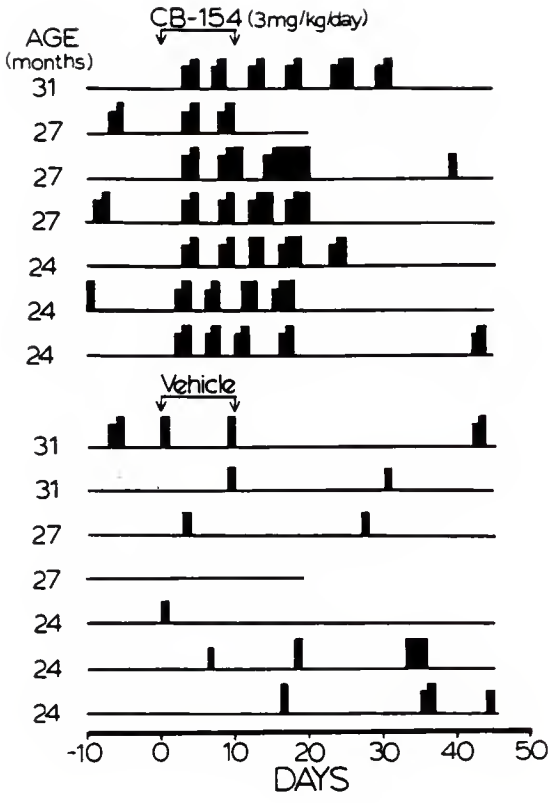

Figure 11. Estrous Cycle Reinitiation in Old Fischer 344 Repeated Pseudopregnant (PP) Rats Treated Daily with CB-154 (3 mg/Kg). Baselines indicate diestrous days while intermediate and high bars represent proestrous and estrous days, respectively. Incomplete profiles were obtained in two animals which died during the post-injection experimental period.

the last drug injection and died 10 days later. The remaining six rats returned to the PP state by 21 days after cessation of CB-154 treatment. The seven vehicle-treated rats showed no evidence of estrous cycle reinitiation. Rather, they had sporatic days with proestrous and estrous sloughed vaginal cell types typical of long term PP rats in our colony. One vehicle-treated rat died nine days after the treatment period.

Discussion. The results of this study clearly show that normal estrous cycles are reinitiated with daily injection of a DA agonist in old PP F344 rats. Since no functional alterations in LH secretion response, LHRH concentration, and maintained or augmented hypothalamic NE activity were observed in these animals, it appears that elevated prolactin maintains the PP state in old F344 rats.

The inability of ovariectomy to reduce serum prolactin levels in PP F344 rats has been previously observed in PP Long-Evans animals[161-175] and is in marked contrast to the substantial reduction of prolactin secretion following ovariectomy in CE rats.[113,117,144,159,161,175] These results indicate that a hypothalamo-hypophyseal deficiency, rather than an ovarian mechanism, is responsible for the persistent hyperprolactinemic state of PP rats. The preceding observation that enhanced tuberoinfundibular DA activity coincided with elevated serum prolactin in the F344 rat suggests that a decline in anterior pituitary responsiveness to the inhibitory influence of DA or another prolactin-inhibiting factor may mediate this consistently observed hypersecretion of prolactin.

That this persistent elevation in serum prolactin levels is a major, if not the sole factor, responsible for the PP state of F344 rats is indicated by the abrupt and consistent reinitiation of normal ovarian cycles following initiation of CB-154 treatment. The luteotropic action of

prolactin is well documented in the young, sexually mature rat. Further, treatment with DA agonists rapidly restores ovarian cyclicity in young, experimentally induced pseudopregnant rats as well as in old PP Wistar rats.[179,259]

Collectively, these results indicate that the hyperprolactinemic state, rather than a disruption of the LH secretory mechanisms, is the primary contributor to the PP reproductive status of old PP F344 rats. The present observation of persistent hyperprolactinemia following ovariectomy together with evidence of hyperactive TIDA neurons in the PP F344 rat suggests that an alteration in pituitary mammotrophs allows elevations in serum prolactin concentrations and hence, the resulting PP state.

GENERAL DISCUSSION

Studies described in this thesis were designed to evaluate the concept that progressive decreases in central CA neuronal activity during advanced age result in impaired neuroendocrine regulation of AP hormone secretion. This etiology has been proposed to explain the cessation of cyclic ovarian function and hyperprolactinemia observed in aged female rats.[113] Numerous studies have provided correlative evidence that impaired CA function may be causally related to the onset of reproductive senescence; however, these studies have not clarified the relationship between CNS alterations in old rats and the apparently different neuroendocrine impairments associated with the CE and PP reproductive states in these animals (see Literature Review Sections: Age-Related Alterations in Reproductive Function and Age-Related Alterations in the Central Nervous System). Therefore, the present studies were designed to extensively characterize the nature of CA neuronal alterations along the preopticotuberal pathway in aging animals prior to and after establishment of the CE or PP states. The effects of increasing age on LHRH concentrations within hypothalamic regions were then characterized in animals which experienced the PP or CE states. In conjunction with this descriptive approach, both LH and prolactin secretion responses were monitored as an index of the extent and nature of neuroendocrine impairments in these aging rats. Studies were then designed to more directly evaluate the contribution of CA systems to the age-related alterations in neuroendocrine function. This latter approach employed pharmacological manipulation of CA systems in old CE and PP rats.

The results of the three studies which characterized the nature of CA alterations during advancing age clearly show that neither uniform or widespread changes in CA concentrations or activity are present in hypothalami of old compared to young rats. Rather, changes are focalized in relatively discrete regions and both the magnitude and direction of these changes vary with the sex, reproductive state and/or strain of animals. Thus, these results do not indicate that progressive decreases in CA neuronal function occur during advancing age. However, these results do not discredit the concept that changes in hypothalamic CA activity contribute to the onset of reproductive senescence and altered neuroendocrine regulation.

Comparison of the pattern of age-related changes in CA activity with LH and prolactin levels measured in these animals suggests that the nature of CA activity during advancing age may determine the extent of altered neuroendocrine function. Results of the initial study in this thesis indicated that augmented prolactin concentrations in old male rats were associated with relatively large decreases in DA concentrations within MBH regions while DA levels were augmented in anterior hypothalamic regions. In contrast, the less severe decreases in NE levels were not correlated with changes in basal LH secretion. However, previous studies suggested that the effects of decreased NE levels might be reflected in the diminished capacity of old male rats to augment LH release in response to several stimuli (see 135). While this evidence suggests a correlation between altered hypothalamic CA function and hormone secretion in old male rats, further studies are required to characterize the nature of this relationship.

Results of the study in F344 rats which are relatively long-lived and enter the PP state late in life show that CA concentrations are

unchanged through middle-age and then generally decrease in many, but not all, regions examined in old animals. However, augmented neuron activities (K) maintain CA turnover rates in many regions of old rats at levels comparable to or greater than those measured in young ovariectomized rats. The mechanisms responsible for the observed increases in CA activity in several hypothalamic regions of old rats are not clear.

A pattern of progressive age-related decrease in DA concentration was seen in only two of 12 brain regions examined in F344 rats, although substantial decreases were found in several regions from old rats. Age-related changes in DA turnover rates did not correlate with changes in DA concentration. Further, the results of this study do not indicate that progressive decreases in DA neuronal function precede the establishment of the PP state in these animals. In contrast, DA turnover rates were augmented in three regions from old versus younger animals and neuron activity tended to increase in five of eight areas from old rats as reflected in rate constants (K).

Elevated circulating prolactin in old F344 rats, which apparently is not influenced by ovarian secretion, may contribute to DA alterations observed in these old ovariectomized animals. Previous studies have shown that chronic experimentally induced hyperprolactinemia results in accelerated DA activity and diminished DA concentration.[94-97] This relationship was observed in old rats in the ME, NIL and NVM while DA rate constants tended to increase in the POAm and striatum. Thus, the changes observed in DA neuronal function in old F344 rats do not indicate that impaired DA activity contributes to the hyperprolactinemia of these animals. Indeed, these results show that DA neurons maintain their capacity to augment activity through advanced age. The hyperprolactinemia observed

in old F344 rats cannot, therefore, be the result of impaired TIDA function, since the turnover rate of DA was significantly augmented in the ME of these animals. Rather, these studies indicate that hyperprolactinemia in old F344 rats may result from alterations in lactotrophs or alternatively, the diminished secretion of another unidentified prolactin inhibiting factor.

The pattern of change in NE neuron function with age observed in seven of eight regions in F344 rats was that increased NE turnover rates precede diminished NE concentrations. This observation suggests that NE hyperactivity may result in neuron "burnout" as reflected in subsequent declines in amine concentration and turnover rate. The time course for these changes was variable among regions examined. Turnover was increased in middle-aged animals in six of eight regions and significantly elevated in the ME of old animals. Further, the effects of decreased NE concentrations on turnover rate in old animals varied between regions. Turnover rates in old rats were dampened compared to young animals in only three of seven regions.

Both the mechanisms responsible for augmented NE neuron activities and the effects of these increases during postmaturational aging in F344 rats are unknown. It is possible that these changes reflect alterations intrinsic to NE neurons such as impaired negative feedback regulation which could be mediated by alterations in presynaptic receptors.[275] Alternatively, changes in other neurotransmitter systems which inhibit NE neuron function could contribute to hyperactivity of NE neurons. Such a role has been proposed for the opioid systems.[276] Reports have indicated that some changes occur during advanced age in both adrenergic receptors[199,228-230] and opioid activity;[277] however, the relationship, including time course, magnitude and interactions of these alterations, has not been extensively studied.

The relationship between NE changes with age and gonadotropin regulation in old F344 rats is not readily apparent. No changes in LH response to ovariectomy were detected between age groups of these animals. This observation indicates that LHRH neurons maintain relatively normal function through advanced age in F344 rats and are not impaired during the PP state. Further, no age-related changes in LHRH concentrations were detected in any region examined in either intact or ovariectomized rats. Thus, the LH secretory mechanisms appear to be relatively unaltered in old PP rats.

The studies in Long-Evans rats, which enter the CE state during the middle third of their median life span, indicate that some progressive decreases in CA concentrations occur with advancing age in the hypothalamic regions examined. This observation is in contrast to the stable levels found through middle-age in the long-lived F344 rat. Although activity (K) of CA neurons tended to increase in some regions of older Long-Evans rats, the magnitude of increase was usually not sufficient to maintain CA turnover rates at levels observed in young animals.

Concentrations of DA were progressively decreased with increasing age in five of nine regions of ovariectomized Long-Evans rats and DA turnover rates were progressively decreased only in the ME and NHA. Significant decreases in DA turnover rate were found in six regions of middle-aged animals indicating that altered DA neuron function precedes establishment of the CE state in Long-Evans rats. Examination of rate constants from old ovariectomized animals shows that DA neuron activity (K) tended to increase in five of six regions which had dampened DA turnover in middle-aged animals. This indicates that DA neurons in these regions maintain their capacity to augment activity but suggests alterations occur in the regulation of DA neuron function with age. It does not appear, from these data,

that DA neurons augment activity to compensate for diminished DA concentrations and thereby maintaining DA turnover rate.

The mechanisms responsible for these differential changes in CA activity during increasing age remain to be determined. They may involve impairments in other neuronal systems which regulate DA neurons or reflect aging of the DA neurons themselves. Specifically, the decreases in DA activity during middle-age may indicate DA neurons tend to decrease function while augmented activities in old animals may result from the concurrent decline in DA concentrations. Whether augmented rate constants in old rats represent response to increased stimulating factors remains to be examined. The possibility exists that alterations occur in both stimulatory and inhibiting components of DA neuronal regulation. It is also possible that prolactin in old CE rats serves to stimulate DA neurons other than those of the TIDA system[94-97] and these stimulatory effects are maintained after prolactin decreases with ovariectomy.

Prolactin levels are chronically elevated in CE rats as shown in the present studies and by others.[113,144] Hyperprolactinemia in the CE rat appears to be maintained by ovarian secretions since castration dampened prolactin in old CE rats to levels observed in young ovariectomized rats. Further, these basal prolactin levels were maintained in old ovariectomized Long-Evans rats in spite of severe age-related decreases in TIDA function. The ability of αMPT to augment prolactin in these animals further indicates TIDA activity suppresses prolactin secretion. Whether these chronic elevations in prolactin in intact CE rats contribute to or accelerate the rate of decline in DA concentration found in several hypothalamic regions of old ovariectomized animals should be investigated. The direction of TIDA neuron change during advancing age differs between the F344 and Long-Evans

rat. The long-lived F344 rat augments TIDA activity during advanced age which may reflect the response of these rats to relative short-term hyperprolactinemia. The CE rat show impaired TIDA function which may reflect the consequences of chronic severe hyperprolactinemia in the aging Long-Evans rat.

In both Long-Evans and F344 rats a positive correlation was shown between the magnitude of NE rate constants (K) and the extent of subsequent decreases in NE concentrations. Thus, results in Long-Evans rats provide additional evidence which indicates NE hyperactivity may result in neuron "burnout." In contrast to the results in F344 rats, no consistent patterns were detected in age-related changes in NE turnover rates or rate constant (K) among hypothalamic regions of Long-Evans rats. Significant differences in NE turnover were detected in five of eight regions in middle-aged animals, indicating that change in NE neuron function precedes establishment of the CE state. While the relationship of these changes to the onset of CE in these animals is unclear, it is apparent that the direction of change in NE turnover during middle-age differs between the Long-Evans and F344 rat. Turnover of NE was augmented in the long-lived F344 middle-aged animal, while NE turnover was unchanged or decreased in middle-aged Long-Evans rats with the exception of the NHA. Whether these changes contribute to subsequent establishment of the CE or PP state is unclear. However, the observation that NE turnover tended to increase in five of eight regions of old compared to younger Long-Evans rats indicates that progressive decreases in NE turnover do not occur after establishment of the CE state.

The role of age-related alterations in NE activity on LH secretion is not clear from these descriptive studies. Postcastration LH secretion

levels were not dampened in middle-aged animals which had severely decreased NE turnover rates in several hypothalamic regions. Additionally, little LH response to ovariectomy was observed in old Long-Evans rats which had less severe decreases in NE turnover rates in some regions. Since decreased NE turnover in several regions appeared to precede establishment of the CE state, additional studies were conducted to characterize the nature of this relationship.

Studies designed to characterize the nature of the postcastration LH response in aging rats show pulsatile LH secretion is severely dampened in old CE rats. While both the frequency and amplitude components of pulsatile LH secretion are decreased in old Long-Evans rats, the diminished frequency primarily appears to contribute to impaired LH response to ovariectomy in these animals. These findings provide evidence that LHRH neurons are impaired in CE rats. In contrast, LH castration response was normal in PP old F344 rats. Extensive characterization of the hypothalamic distribution and concentration of LHRH showed no age-related change in LHRH levels in either Long-Evans or F344 rats. Further, no age-related change was detected in LHRH depletion response to ovariectomy in either animal model. However, the different postcastration LH response between CE old Long-Evans and PP old F344 rats cannot be attributed to AP alterations since repeated LHRH administration resulted in normal LH release profiles in CE Long-Evans rats.[127] Collectively, these results indicate that LHRH neurons maintain relatively normal function through advanced age in F344 rats, but LHRH neuronal activity is impaired in old CE rats. Although LHRH concentrations were stable in old Long-Evans rats, this does not indicate these neurons maintain normal activity. Evidence showing subneuronal LHRH distribution is altered in old CE rats[232] indicates that impaired

release of the neurohormone necessarily should not be accompanied by altered LHRH concentrations. Additionally, these data indicate that monitoring postcastration LHRH depletion may not be an adequate method to evaluate LHRH neuronal function.

The severely dampened pulsatile pattern of LH secretion characterized in ovariectomized old CE rats closely resembles LH secretory profiles observed in young ovariectomized rats following blockade of NE synthesis. Therefore, the ability of an α-adrenergic agonist, CLON, to restore pulsatile LH release after blocking NE synthesis was examined in young ovariectomized rats. Results of these studies showed that adrenergic stimulation appears to play a permissive role to facilitate LHRH neuron function. Since a single injection of CLON stimulated pulsatile LH release, it appears that NE neurons do not drive LHRH neurons to release neurohormone but, rather, facilitate their pulsatile activity.

The ability of CLON to restore pulsatile LH release in middle-aged ovariectomized rats which had briefly experienced the CE reproductive state provided direct evidence that central adrenergic neuronal functional changes contribute to the onset of the CE state. Administration of CLON was unable to elicit pulsatile hormone secretion in old CE rats two weeks following ovariectomy. Whether this reflects progressive impairments in adrenergic receptor, LHRH neuron dysfunction, and/or the cumulative effects of prolonged estrogen exposure which may alter these and other neurotransmitters[149] is at present uncertain. However, these studies provide strong evidence for an adrenergic etiology in the onset of the CE state and associated impaired LH response to ovariectomy and to stimulatory regimens of gonadal steroids. Since young animals can regain normal ovarian cycles and pulsatile postcastration release patterns within weeks after chronic NE

depletion,[76] the present studies may suggest that the CE rat is less able to adapt to impaired NE function. Whether this results from alterations in regulation of other neurotransmitter systems should be investigated.

Results of the descriptive studies in PP old F344 rats suggested that DA neuron changes reflected the responses seen in experimentally induced hyperprolactinemia. Further, the normal postcastration LH response indicated that relatively normal LHRH neuron function is maintained in old F344 rats. Since prolactin is known to maintain pseudopregnancy in young animals,[7] the ability of a DA agonist, CB-154, to restore cyclic ovarian function in old PP rats was examined. The results of this study clearly show that the alteration resulting in the PP state in F344 rats is hyperprolactinemia. Further, elevated prolactin in these animals does not appear to result from impaired TIDA function since ME turnover of DA was augmented in these rats. Whether the mechanisms responsible for hyperprolactinemia in F344 rats involve altered lactotroph DA receptors, a decrease in another prolactin inhibiting factor, or an increase in prolactin release factor remains to be studied. It is apparent from this study that the F344 rat maintains unimpaired gonadotropin regulation through advanced age.

Prior to the initiation of studies described in this thesis, the vast majority of studies attempting to identify alterations responsible for age-related impairments in regulation compared only young and old subjects. Further, the majority of studies investigating neuronal function only reported changes in concentration of neurotransmitters, enzymes or receptor binding activity (see Literature Review Section: Age-Related Alterations in the Central Nervous System). Little consideration was given to the health status of the animals employed in these studies or to their prior reproductive history. While these studies were invaluable in identifying

changes with age, by their design they could not attempt to identity the mechanisms for these alterations. Correlative evidence resulting from these studies suggested that neuronal alterations were progressive in nature and were causally associated with impaired gonadotropin secretion and elevated prolactin levels found in old animals. Supporting evidence for this concept was provided from a few studies which noted CA turnover rates were decreased in old versus young rats in hypothalamic regions.[215,216]

It is apparent from the results of studies described in this thesis that no uniform neuronal alterations occur within hypothalamic regions of old rats. Further, the changes found are usually not progressive in nature. While the results of studies described herein raise more questions than they answer, they clearly indicate that no widespread generalized changes in CA function can explain age-related changes in neuroendocrine function. Future studies to clarify the mechanisms responsible for the often observed age-related decreases in CA concentration should therefore consider several factors. Among these are: animal model, neural region, health status, and the patterns of change during the aging process. It is also apparent from these results that several regulating factors may change at different rates, thus, contributing to the observed alterations.

In summary, these studies showed that no generalized progressive decreases in DA or NE function accompany the aging process. Age-related changes in CA activity are focalized within regions of the hypothalamus and differ in both magnitude and direction within and between the animal models examined. Concentrations of CA generally decrease during advanced age in both F344 and Long-Evans rats. In contrast, turnover rates are maintained or augmented in several hypothalamic regions as a result of increased CA activity (K) in older, compared to younger, animals. This

occurred more frequently in F344 than Long-Evans rats and may be associated with the greater longevity of F344 rats. The results of these studies did not indicate that age-related CA alterations within a specific hypothalamic region or regions are responsible for the onset of the CE or PP reproductive state.

The second aspect of the studies in this thesis evaluated the contribution of CA alterations on impaired hormone regulation in the PP and CE states. Hyperprolactinemia appears to maintain the PP state and does not result from dampened TIDA activity. Rather, TIDA neuron function was elevated in F344 PP rats. Impaired LH castration response in CE rats results from diminished LHRH neuronal activity as reflected by diminished pulsatile frequency and amplitude of LH secretion in these rats. A single injection of CLON can reinitiate pulsatile LH secretion in both young ovariectomized rats after acute NE depletion and in ovariectomized middle-aged CE rats which have dampened NE activity in several hypothalamic regions. Thus, a NE dysfunction appears to mediate the onset of the CE state. The inability of CLON to stimulate LH in old rats which had experienced CE for months may indicate progressive impairments occur in LHRH neuronal function or that a substantial decline in adrenergic receptor responsiveness occurs with prolonged constant estrus.

It is hoped that the results of studies described herein will provide a basis for future investigation of the mechanisms responsible for the multiple patterns of neuronal alteration presently characterized. The evidence that hormonal secretion can be restored in aged animals with pharmacological manipulation of the CA systems suggests that similar approaches may eventually be applied to several neuroendocrine impairments which accompany advanced age.

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BIOGRAPHICAL SKETCH

Kerry S. Estes was born in Lansing, Michigan, on March 30, 1952. She graduated from Waverly High School in 1969. She then enrolled at Kalamazoo College where she received her B.A. in 1973 with a major in biology. During this time, her interest in reproductive endocrinology was stimulated as a result of her experience as a "normal volunteer patient" in the Endocrinology Unit at the NIH in the Spring of 1971. This interest was then focused to research through the guidance of John Wilks in the Summer of 1972 at the Upjohn Company. He directed her "Senior Individualized Project" entitled "Influence of the Thyroid Gland on the Onset of Puberty in the Female Rat". She then began studies with Edward M. Convey in the Physiology Department at Michigan State University where she received the M.S. Degree in 1976 with a thesis entitled "Localization of Gonadotropin Releasing Hormone Within the Bovine Hypothalamus". In 1977, she was attracted to the University of Florida. There she began her dissertation work with James W. Simpkins in the College of Pharmacy. She completed studies for her Ph.D. in 1982. She married Hartmut Derendorf in September, 1982.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

James W. Simpkins, Chairman
Associate Professor of Pharmacy

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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